University of Alberta

Impacts of multiple anthropogenic stressors on boreal planktonic communities

by

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ABSTRACT

Boreal aquatic systems are subject to the cumulative impacts of global warming, drought, and acidification. Interactions amongst these stressors have produced more detrimental abiotic conditions than expected, but their biological impact is largely unknown. To test the hypothesis that these stressors interactively affect biota, an *in situ* mesocosm experiment was performed in a recovering acidified boreal lake (Lake 302S, Experimental Lakes Area, Canada). Results showed that interactive effects were common, causing highly unpredictable changes in community composition and total biomass. To test the hypothesis at larger spatial and temporal scales, I analyzed the 23-yr history of producer and consumer communities in Lake 302S. My mesocosm-based findings were corroborated by whole ecosystem-level evidence from Lake 302S, which showed that non-additive effects of environmental stressors regulated the biomass of planktonic consumers and producers. I conclude that interactions amongst multiple stressors will shift boreal aquatic ecosystems along complex and unknown future trajectories.

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CHAPTER ONE

CUMULATIVE IMPACTS OF MULTIPLE ECOLOGICAL STRESSORS: ADDITIVE OR NON-ADDITIVE EFFECTS?

INTRODUCTION

Global change is defined by the cumulative impacts of multiple anthropogenic stressors on biodiversity and ecosystem function (Sala et al. 2000; Steffen et al. 2004). For example, modern changes in northern landscapes are attributed to the "triple-whammy" effects of acid deposition, climate warming, and stratospheric ozone depletion (Gorham 1996; Schindler 1998). Coral reefs are being altered by the combined influence of climate change, pollution, over fishing, and other factors (Hughes et al. 2003). Forest decline and loss of grassland diversity are being attributed to the interactive and direct effects of several stressors, such as climate change, and elevated carbon dioxide (CO_2), nitrogen deposition, and tropospheric ozone (Aber et al. 2001; Reich et al. 2001; Shaw et al. 2002; Zavaleta et al. 2003). The impacts caused by floods and runoff are intensified in catchments disturbed by forest fires (Johansen et al. 2001; Bouchon and Arseneault 2004; Vieira et al. 2004). Similarly, multiple introductions of exotic species are causing "invasional meltdowns" of terrestrial and aquatic ecosystems (Simberloff and Von Holle 1999; Riccardi et al. 2001). Across large scales and a wide diversity of stressors, it is becoming increasingly apparent that various combinations frequently generate 'ecological surprises' (*sensu* Paine et al. 1998).

The cumulative impact of multiple ecological stressors often does not equal the sum of their reported individual effects, which confounds ecological risk assessments (Heugens et al. 2001). A non-additive ecological impact is the consequence of interactions among the different stressors. These interactions cause the net impact to either synergistically exceed or antagonistically fall short of the expected additive effect (Fig. 1.1). Consequently, single-stressor studies

cannot be used to forecast the cumulative impact of interactive ecological stressors. For example, Sala et al. (2000) concluded that interactions among anthropogenic stressors are the major source of uncertainty in models predicting future changes in global biodiversity.

In this review, I hypothesize that non-additive impacts of multiple stressors are more prevalent than additive effects in nature owing to ecological trade-offs (Kneitel and Chase 2004), stress-induced tolerances (Blanck 2002), and the non-linearity of species responses along stress gradients (i.e. Gaussian response curves). For instance, correlated biological traits that affect species co-tolerance should amplify or suppress the net impact of multiple stressors beyond that expected based on the sum of their individual effects (Vinebrooke et al. 2004). Differential effects of stressors across trophic levels (Vinebrooke et al. 2003; Raffaelli 2004) should further confound additive effects by affecting trophic interactions. Nonetheless, the accelerating multiplicity of unique combinations of anthropogenic stressors highlights the increasing probability of complex interactions among ecological stressors.

The nature of interactive effects (i.e. synergism versus antagonism) by multiple stressors will also expectedly vary among trophic groups. Specifically, I hypothesize that multiple stressors are more likely to exert synergistic impacts on top predators (e.g., vertebrate carnivores), and antagonistic effects on lower trophic groups (e.g., microbes and primary producers). My rationale for this hypothesis stems from differences in species richness, dispersal potential, physiological acclimation, and evolutionary history and adaptation that exist between microbes, invertebrates, and vertebrates. These same traits also expectedly affect how risk of non-random loss of biodiversity differs among trophic levels (Raffaelli 2004).

TYPES OF ECOLOGICAL STRESSORS AND THEIR INTERACTIONS

I define the term "stressor" as a relatively novel abiotic or biotic environmental variable that exceeds its range of normal variation as a consequence of human activity. A stressor elicits a statistically detectable "stress" response that involves a biological process or aggregate property (e.g., species richness) being significantly displaced from its natural state (sensu Odum 1985). Therefore, although stressors are typically considered as being negative (e.g., high acidity, heavy metals, pesticides), certain stressors can instead exert positive ecological effects (e.g., eutrophication; see Cottingham 1999; Klug and Cottingham 2001). Similarly, others have used the term *disturbance* to denote a distinct environmental event that disrupts ecosystem properties over space and time (e.g., Pickett and White 1985; Paine et al. 1998).

A simple additive model provides a starting basis for defining the cumulative impact of multiple ecological stressors. Here, the net impact of several stressors does not differ significantly from the sum of their individual direct effects (i.e. no interaction). In other words, simultaneous or consecutive exposures to multiple stressors will have the same net ecological impacts as expected based on the sum of their individual effects.

Additive effects are typically attributed to each stressor affecting distinct biological traits that is unaffected by the other stressors (Folt et 1999; Vinebrooke et al. 2004). For example, Reich et al. (2001) showed that elevated CO_2 and nitrogen exerted a combined additive effect on plant biomass because of their independent effects on below- and above-ground biomass, respectively. Similarly, an additive model best explained the combined impact of elevated CO_2, nitrogen, precipitation, and warming on grassland diversity (Zavaleta et al. 2003), possibly also because of they each altered a different aspect of resource availability. Additionally, a net additive effect of low oxygen and effluent on grazing pressure in streams resulted from the direct effect of the worst stressor, namely anoxia (Lowell and Culp 1999).

Non-additive models are defined by interactions among stressors in which the ecological effect of one stressor is dependent upon another (Fig. 1.1). As a result, the net ecological impact of interactive stressors will differ significantly from their additive effect. Non-additive effects may result from interactions among stressors occurring at either the environmental (exogenous) or organismal (endogenous) level. An example of an exogenous interaction is when ultraviolet radiation (UV) directly increases the toxicity of chemical pollutants via photoreactive mechanisms (Bell et al. 2004; Blaustein et al. 2003). Alternately, endogenous interactions can be demonstrated among exothermic organisms having higher UV repair rates under warmer temperature (Williamson et al. 2002). Regardless of precise mechanisms, interactions among environmental stressors will generate non-additive ecological impacts.

The synergistic non-additive model involves a stressor amplifying the effect of another, generating a net ecological impact that is significantly greater than the sum of their individual effects. Synergistic interactions among stressors often involve the ecological mechanism termed stress-induced sensitivity (Vinebrooke et al. 2004). In other words, exposure to one stressor lowers the threshold of an ecological response to other stressors. For example, pesticides weakened the resistance of tadpole populations against a seemingly harmless caged predator (Relyea and Mills 2001). Similarly, pesticides cause zooplankton to lack anti-predator strategies, thereby amplifying the effect of predation (Hanazato 2001). Predation, pesticides, and metals also intensify the marginal effects of hypoxia on benthic invertebrates (van der Geest et al. 2002). Fertilization of grassland communities increases their sensitivity to drought by competitively extirpating stress-tolerant plant species (Tilman and Downing 1994). Further, the 'invasional meltdown' hypothesis (Simberloff and Von Holle 1999) typifies a synergistic scenario in which introduction of a species facilitates colonization by another exotic species, thereby amplifying their net effect on native species.

Antagonistic non-additive models operate via ecological stressors suppressing each other, resulting in a combined impact that is significantly less than their additive effect. An explanation for antagonistic interactions among ecological stressors involves exposure to one stressor inducing physiological resistance or genetic tolerance to other stressors (Blanck 2002; Vinebrooke et al. 2004). For example, elevated temperatures increased the salinity tolerance of coral (Porter et al. 1999). Similarly, warming dampened the impact of grazing on plant species richness (Klein et al. 2004). Other antagonistic interactions are modeled solely on the expected maximum effect of the most dominant stressor (e.g., Sala et al. 2000). Folt et al. (1999) termed this a 'simple comparative scenario,' equating it to Liebig's law of the minimum in which the ecological impact of the greatest stressor negates the effects of others.

STATISTICAL ANALYSIS OF MULTIPLE ECOLOGICAL STRESSORS

My use of the term "interaction" corresponds with its statistical definition as used in analyses of variance (ANOVA). ANOVA is commonly used by ecologists (Cottingham et al. 2004), but appears to be often misinterpreted regarding the importance of interactions and main effects. In any multi-factorial ANOVA, the highest-order interaction that is significant merits interpretation, while all lower-order interactions and main effects should be considered statistically irrelevant despite possibly significant *p*-values (Zar 1999). Otherwise, reporting main effects when their interactions are deemed significant following a statistical test is problematic because an ANOVA pools results together, creating meaningless and potentially incorrect conclusions concerning direct effects. However, separate inspections of lower-order sources of variation are still necessary to determine the nature of a significant interaction (i.e. antagonistic versus synergistic).

To help illustrate this point, I refer to a subset of Persaud's and Williamson's (2005) mesocosm experiment, the abundance of *Ploesoma truncatum* under reduced temperature and UV (Fig. 1.2; Table 1.1). Examination of the graph

suggests that *P. truncatum* was not affected by removal of UV under ambient temperature, but had greater abundances under cooler temperatures. Combination of cooling and UV had no impact relative to the control, indicative of an interaction. However, ANOVA reported that both main effects and the interaction were highly significant. Thus there appears to be discrepancy between the graph and the ANOVA table.

In ANOVA reporting UV is *not* simply the significance of the main effect of UV; rather ANOVA tests for the effect of UV, *irrespective* of temperature. Thus to test for the effect UV, ANOVA pools together experiments where UV was shielded (-UV and cold/-UV) and compares it to the UV controls (control and cold). Examining the graph in this light, we can see how ANOVA determined that UV was significant (compare white vs. black bars). Persaud and Williamson (2005) were correct in ignoring the main effects when a significant interaction was detected, otherwise they would have drawn the incorrect conclusion that UV was significant. Additionally, it makes little sense to test for the significance of UV irrespective of temperature when it was shown that the effect of UV is temperature dependant (i.e. UV x Temp interaction).

Although Persaud and Williamson's (2005) methodology was correct, and recommended by statisticians, disregarding information is hardly ideal, as principle effects and simpler interactions are still of great importance. To solve this dilemma, I propose running 'protected ANOVAs' when significant interactions are detected. Using the above example, the significance of the main effect of UV can be tested by using only data from the controls and UV alone (i.e. a *T*-test). Using this method reveals that the main effect of UV was not significant ($P = 0.351$), but temperature was ($P = 0.006$), which matches the original predictions based upon graphical interpretation.

This solution can also be expanded to suit 3-way (or greater) ANOVAs. Imagine a 3 factor experiment testing the effects of A, B and C in a 2 x 2 x 2

design. A significant A x B x C interaction can distort conclusions of the main effects and two-way interactions. Thus a 2-way 'protected-ANOVA' using the controls, A, B and A x B treatments as data can be used to determine the significance of the AB interaction.

Reporting the results from running several protected ANOVAs should look identical to a regular ANOVA table, with asterisks denoting which terms were calculated using protected ANOVAs.

CUMULATIVE EFFECTS AND CORRELATED SPECIES TRAITS

Ecological trade-offs

Traits that allow a species to become successful under certain conditions typically have trade-offs under other circumstances (Kneitel and Chase 2004). For example, the ability to tolerate stress is typically negatively correlated with competitive ability (Tilman and Downing 1994; Hessen 1996; Graham and Vinebrooke 1998; Liancourt et al. 2005) or colonization potential (Suding et al. 2003; but see Emery et al. 2001). Another trade-off many species encounter is 'seed' size vs. 'seed' number; larger offspring have greater rates of survival, but producing many small progeny ensures that at least a few persist, additionally increasing the probability of wide dispersal (Turnbull et al. 1999). Strategies used to avoid stress typically result in increased susceptibility to predation (Jacob and Brown 2000; Hanson 2000; Boeing et al. 2004).

Ecological trade-offs are akin to synergistic interactions among stressors; the ability to tolerate a single stressor enhances the effect of subsequent stressors, thus the net effect of two stressors is greater then expected. For example, hypoxia causes aquatic invertebrates to increase ventilation rates; however, this response can cause increased uptake of toxic compounds (van der Geest et al. 2002; Lenihan et al. 2003). Similarly, biota demonstrate heightened toxicity to compounds under warmer temperatures (Heugens et al. 2003) or the

presence of predators (Hanazato and Dodson 1996; Relyea and Mills 2001). Lafferty and Holt (2003) model environmental stress as having reduced resistance to non-specific diseases. Experimental results have demonstrated that ultraviolet radiation (UV) can increase mortality by having negative synergistic interactions with warming and salinity for marine gastropod eggs (Przeslawski et al. 2005), nutrient limitation among phytoplankton (Xenopoulos et al. 2002) and for tadpoles in eutrophified ponds (Hatch and Blaustein 2003). In each of the above examples, biota responded synergistically, either by being overwhelmed by simultaneous exposure to multiple stressors, or because traits used to cope with one stress enhanced the effectiveness of the other. If ecological trade-offs predominate in nature, I predict that the net effect of any two stressors would result in synergistic interactions.

Ecological co-tolerance

In contrast to ecological trade-offs, many species exhibit co-tolerance or stress-induced tolerance when exposed to multiple stressors. Co-tolerance is defined as having positively-correlated species traits (i.e. high tolerance to two separate stressors; Vinebrooke et al. 2004). Co-tolerance typically occurs when mechanisms used to avoid one form of stress are effective in reducing impacts from others. Individuals can achieve co-tolerance via acclimation or adaptation (Djawdan et al. 1997) as can communities through species turnover (Courtney and Clements 2000). For example, a shift toward smaller body size is a typical response to stress (Odum 1985; Moore and Folt 1993; Catteneo et al. 1998; Rapport et al. 1998); therefore, communities exposed to one stress may already be pre-adapted to other impacts.

Ecological co-tolerances result in antagonistic interactions among stressors. Positively correlated species traits and stress-induced tolerances suggest that biota exposed to one stress will suffer minimal impacts from subsequent stressors. Drought, low temperatures and UV each cause northern conifers to

develop smaller thicker leaves; thus, the presence of one stress can protect individuals from the effects of others (reviewed in Turunen and Latola 2005). Warmer temperatures increase the rate of photo-enzymatic repair in some zooplankton (Williamson et al. 2002) intertidal algae (Hoffman et al. 2003) and aquatic bryophytes (Núñez-Olivera et al. 2004), resulting in increased UV tolerances. Co-tolerances are likely to occur among functionally similar stressors; for example tolerance to multiple salts among grasses (Kobayashi et al. 2004) and tolerance to various metals among plants (Schat and Vooijs 1997), periphyton (Soldo and Behra 2000) and microbial communities (Blanck 2002). Antagonistic interactions suggest that individuals, communities and ecosystems could potentially be more resistant to the impacts of multiple stressors then predicted by additive models.

It is important to note that while trade-offs and co-tolerances can explain synergistic and antagonistic interactions respectively, they are not the only sources of non-additivity. An organism can have high tolerances to two stressors when each are experienced in isolation, but exogenous interactions can cause heightened levels of stress. Similarly, if tolerance to each stressor occurs via separate mechanisms, individuals may have tradeoffs in energy allocation and thus fail to fully operate both mechanisms (Hanazato and Dodson 1995). The response of other trophic levels may also determine whether organisms respond according to additive models, and will be discussed further below.

SCALE- AND CONTEXT-DEPENDENCY

While it is exceedingly difficult to predict the occurrence and direction of 'ecological surprises', I suggest that inherent ecological characteristics of communities should largely dictate their response to multiple stressors. Specifically, species diversity, physiological and genetic adaptation, and dispersal potential, particularly in their relation to trophic position, are of prime

consideration. I provide a theoretical diagram that hypothesizes the impacts of multiple stressors on ecosystem function (Fig. 3)

This simplistic ecosystem is comprised of three trophic levels: predators, grazers and producers. Individual species within each trophic level are denoted by a single point. The location of this point indicates a species' tolerance to both stressor A and B (i.e. a species located in the top right corner has high tolerance to both stressors, while a species located in the bottom left corner has a low tolerance to both stressors). Stressors A and B represent any stressor that has the ability to cause extirpation of a species either immediately through direct mortality, or gradually through reduced fitness. Species located within the shaded regions are considered 'sensitive' and are thus extirpated upon the application of either stressor A or B, while species in the un-shaded regions are termed 'tolerant' of multiple stressors. Assuming that species respond to stressors A and B according to the additive model, the presence of stressor A causes the left side, and stressor B causes to bottom of the box to become shaded. This diagram is an expanded view of an earlier concept (see Vinebrooke et al. 2004). The following sections will relate this diagram and I will demonstrate how differences between trophic levels can dictate community response to multiple stressors.

Diversity and Compensatory Species Dynamics

Biodiversity has an important role in buffering ecosystem function in fluctuating and stressed environments (Petchey et al. 1999; Naeem and Li 2000). High richness increases the likelihood that tolerant species will be present and will compensate for the loss of sensitive species, thereby maintaining net function (Ives et al. 1999). Higher trophic levels typically have low inherent diversity and are thus less resistant to stressors and local extinction (Vinebrooke et al. 2003; Petchey et al. 2004; Raffaelli 2004). In Fig. 3, we model the predator community as has having only three species, two of which were eliminated due to the

presence of multiple stressors. I suggest that a single predatory species is typically incapable of maintaining net predation due to the prey selectivity of most predators, and the absence of positive predator interactions (Losey and Denno 1998). Although both herbivores and producers lose substantial numbers of species under the application of multiple stressors, I suggest that they retain enough species to uphold community function. Biodiversity is likely a key determinant in the response of communities to multiple stressors, with more diverse communities (as typically found in lower trophic levels) being increasingly resistant. Extremely low-diversity systems (i.e. monocultures) could be extremely susceptible to both single as well as multiple stressors.

Flexibility and Adaptability

In addition to the reduced diversity of predatory communities, higher trophic levels are generally considered to be less tolerant of environmental perturbations. Microbial communities have been demonstrated as having greater tolerance to temperature and ultraviolet radiation then their grazers (Bothwell et al. 1994; Rae and Vincent 1998; Strecker et al. 2004; Winder and Schindler 2004). Additionally lower trophic levels typically have shorter generation times and rapid turn over rates and are thus under strong evolutionary pressures to adapt to stressful conditions (Fischer et al. 2001; Raffaelli 2004).

In addition to their higher inherent tolerance of single stressors, lower trophic levels are also more likely to display co-tolerances among multiple stressors, while trade-offs should be demonstrated among higher trophic levels. Microbial communities when exposed to one stress generally appear to have higher tolerances to a suite of other stressors resulting in antagonistic interactions (Arzumanyan et al. 2002; Blanck 2002). Conversely, fish and amphibian populations seem exceedingly susceptible to synergistic interactions resulting in high levels of mortality (Power 1997; Blaustein and Johnson 2003; Chen et al. 2004). Likewise, a lack of stress-induced tolerances were found

among food deprivation and insecticide in beetles previously exposed to heavy metals (Stone et al. 2001)

To illustrate that lower trophic levels are typically more tolerant to perturbations, I drew primary producers as having a high degree of positive co-tolerance between stressors (see Vinebrooke et al. 2004). Primary producers also had the greatest range of tolerances. Lastly, producers are more likely to exhibit antagonistic interactions, thus species predicted to be eliminated, might be found under multiple stressor scenarios. I graphically represented antagonistic interactions by reducing the size of the shaded region, thus less species are eliminated then predicted. In contrast, predators were drawn as having trade-offs (negative co-tolerances), low tolerances and synergistic interactions (large shaded region).

Regional considerations

The response of a community is not only dependent upon the species within the impacted area, but also those external to the region. Meta-communities can posses highly different species assemblages through differences in biotic and abiotic conditions (Hanski 1998 and sources within) and may thus act as source of tolerant species. For example, pristine systems are less likely to contain stress-tolerant individuals, but after a perturbation, may be successfully colonized from surrounding perturbed areas. Thus, the regional diversity could be more important in determining the ultimate response of a community then local diversity. Large beta-diversities increase the odds that tolerant individuals are present that could potentially colonize disrupted systems.

Potentially more important than regional diversity, is the ability of species to disperse and colonize adjacent areas. Microbial communities are assumed to be ubiquitous, and thus freely colonize (Finlay et al. 1999). Plants have a wide variety of dispersal mechanisms, although they can still be considered dispersal-

limited, particularly over large distances and barriers (Ehrlen and Eriksson 2000; Soons et al. 2005). Although some invertebrates have remarkable dispersal abilities, (e.g. the monarch butterfly) other species can suffer from dispersal limitation (Bohanak and Jenkins 2003). Vertebrates, despite their high motility, can suffer dispersal limitation due to habitat fragmentation and lack of dispersal corridors (Magnuson 1976; Aurambout et al. 2005 and sources within). I visually depicted the role of colonization as the supplement of additional stress-tolerant individuals. Colonization potential is the highest among lower trophic levels due to high regional diversities and dispersal mechanisms. The opposite is true for higher trophic levels.

Temporal Responses

The impacts of multiple stressors will also have large temporal considerations. As earlier life-stages are typically more sensitive to stress then mature stages (Stohlgren et al. 1998; Leech and Williamson 2000; Hoffman et al. 2003; Prezeslawski et al. 2005), the annual timing of stress events can have radically different impacts on communities. The timing of year at which global warming occurs will ultimately dictate how many organisms will respond to UV (Williamson et al. 2002). The ecological history (*sensu* Fischer et al. 2001) of an ecosystem will also determine the response to environmental stressors. Stressors to which there has been no prior exposure are likely to have large impacts due to the abundance of stress-intolerant species and the lack of evolutionary adaptation among tolerant individuals. Courtney and Clements (2000) showed that communities were highly tolerant to a previously exposed stressor, but possessed little resilience to a novel one. Tolerance to the previously exposed stressor occurred at the community level (shift to tolerant species) and population level (marginally sensitive species evolved higher tolerance). Similarly, Fischer et al. (2001) found that communities that had been previously acidified were remarkably more tolerant to re-acidification, partially due to evolutionary adaptation.

Trophic Considerations

Lastly, I consider the role of trophic interactions in dictating the response of communities to multiple stressors. I argue that higher trophic levels should be disproportionately impacted, thus trophic cascades should occur. In my model ecosystem, multiple stressors should largely release grazers from predation while not significantly hindering their productivity. Thus, increased grazing upon producers should limit primary production despite their relative resistance to multiple stressors. Obviously this view of a trophic cascade is overly-simplistic and does not account for the presence of omnivory (Strong 1992; Vadeboncoeur et al. 2005), intraguild predation (Polis and Holt 1992; Snyder and Wise 2001), edibility of producers (Strong 1992; Ghadouani et al. 2003), or nutrient-poor conditions (Benndorf et al. 2002), which otherwise prevent trophic cascades. Interactions among multiple stressors may also cause bottom-up effects that impact higher trophic levels. The interactive effects of increased nitrogen deposition, warming, CO_2 and precipitation had non-additive impacts upon plant litter quality (Henry et al. 2005). Similarly, reduced light and nutrient enrichment had interactive effects on algal nutritive quality and subsequent grazer biomass (Urabe et al. 2002). Interactions among multiple stressors likely have complex top-down and bottom-up effects, further complicating predictions at both community and ecosystem scales.

CONCLUSIONS

A large focus of previous and current research has been upon the impacts of a single stressor; however, interactions among multiple stressors generate non-additive impacts, may render predictions based on single-stressor data to be of minimal use. Given the large number of potential stressor combinations, and that non-additive effects appear to be more prevalent then additive ones, it is proposed that future global change will be largely dictated by non-additive effects. Higher trophic levels should display largely synergistic interactions

among stressors due to their low physiological flexibility, diversity, and dispersal mechanisms. In contrast, lower trophic levels should be more resistant to multiple stressors and potentially respond via antagonistic interactions. However, more study in this area is clearly required. I propose that increasing numbers of field and laboratory experiments explore the effects of non-additive responses. I also suggest that researchers clearly indicate their research as examining the impacts of multiple stressors. Many of the examples of multiple stressors within this review could not have been found using key words such as 'multiple stressors, non-additive, or interaction.' Researchers also need a greater level of understanding of the use of ANOVA's and other statistical techniques

THESIS OBJECTIVES

The primary objective of this thesis is to determine if climate change and acidification have either additive or non-additive effects on boreal planktonic communities. As identified above, non-additive impacts imply that ecosystems may be significantly more fragile or resilient then previously expected. Interactions among climate variables and acidification are also highly relevant due to their large geographical overlap, and that they interact at abiotic and possibly biotic scales. This objective was achieved by corroborating data from a long-term, ecosystem-scale experiment and a short-term mesocosm experiment, both of which experienced similar multiple stressor scenarios.

The secondary objective of this thesis is to integrate and summarize the role of multiple stressors across all ecosystems and trophic levels. There is a general lack of consensus and understanding of interactions among multiple stressors that requires a review of the literature and presentation of hypotheses. Chapter one attempts to provide a common understanding of the subject, such that further research can more accurately investigate non-additive effects.

Chapter two focuses on the impacts of multiple stressors on planktonic communities and their individual member species to compare and contrast their responses across trophic levels. Further support for these results is gained by examining the response of biota in Lake 302S. The role of generalist vs. specialist species is discussed in a multiple stressor and biodiversity context.

Chapter three investigates the response of an aggregate property (total biomass) of communities to multiple stressors at two spatial-temporal scales, centering on Lake 302S at the Experimental Lakes Area, Canada. The results of the mesocosm study that tested the interactive effects of temperature, DOC and pH on zooplankton and phytoplankton biomass are presented. To provide further support and greater application of the mesocosm experiment, biota from the 23-yr history of Lake 302S are related to the same abiotic factors to determine if interactions occur at the ecosystem scale.

Chapter four provides a summary of thesis findings, and integrates the results from the function and diversity chapters. Lastly, the chapter offers further research opportunities in exploring the role of multiple stressors in aquatic ecosystems.

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Table 1.1. Results from ANOVA, determining the significance of UV, temperature and their interaction on the abundance of *P. truncatum*. Modified from Persaud and Williamson 2005. ANOVA results suggest that all terms and interactions are significant; however, examination of the graph (Fig. 1.2. suggests that UV should not be significant)

Source	SS	df	MS	F	p
UV	10726.9	1	10726.9	19.49	0.001
Temp	5587.2	1	5587.2	10.15	0.01
UV x Temp	6732.1	1	6732.1	12.23	0.006
Error	6055.7	11	550.5		

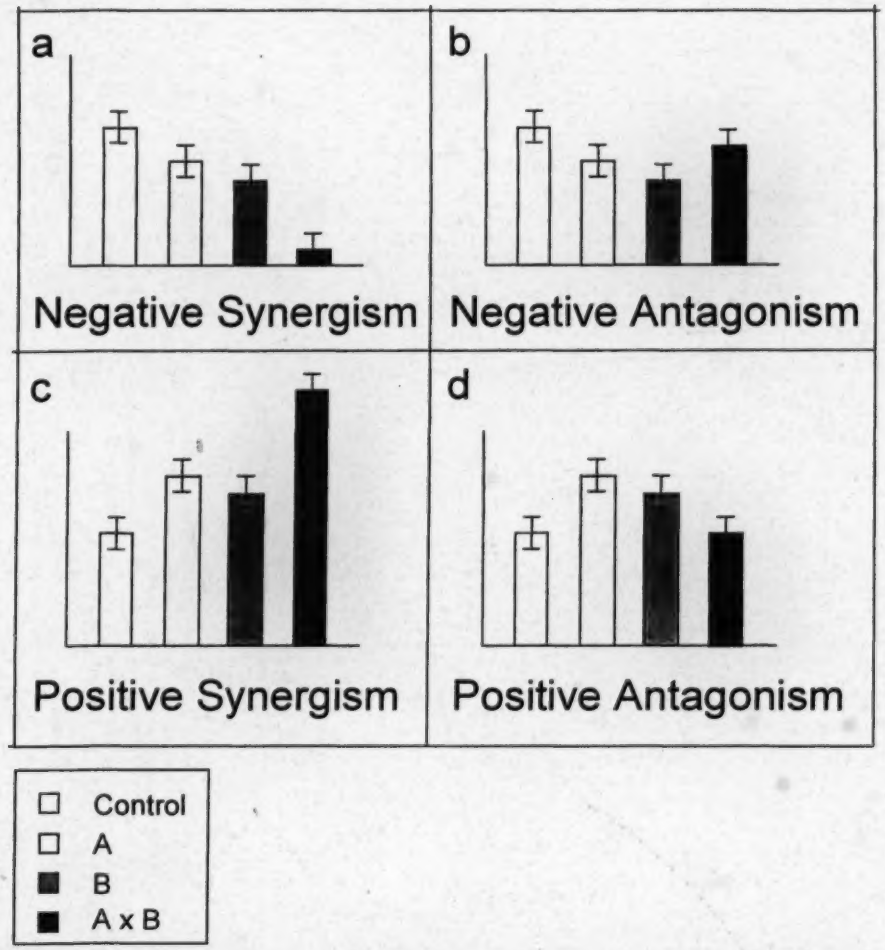

Fig 1.1. Visualization of interactive effects, as demonstrated by providing four potential results from a two-factor experiment involving stressors A and B. Y-axes represent any individual, population, community or ecosystem response (e.g. respiration, productivity, diversity, biomass etc.) Typically, stressors are seen as having negative effects, as shown in **a)** and **b)**, although positive stressors do occur, shown in **c)** and **d)**. **a) Negative Synergism** - combination of A and B is more detrimental than predicted from A and B alone. In contrast, **b) Negative Antagonism** - the interaction between A and B has an ameliorating effect, resulting in a smaller decrease than expected. **c) Positive synergism** - the interaction between A and B is beneficial, resulting in a greater increase then expected. **d) Positive antagonism** – Although A and B were favorable alone, their interaction is harmful, resulting in a smaller effect then expected.

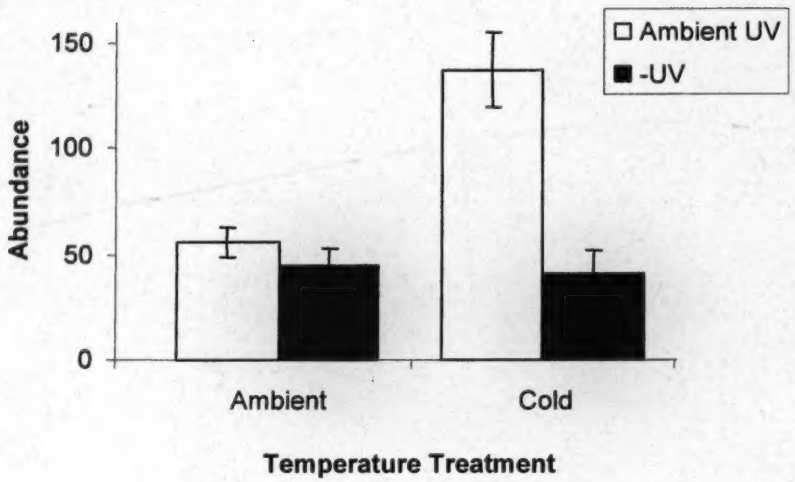

Fig. 1.2. The effect of UV and temperature on the abundance of *P. truncatum*, demonstrating the problem of determining the significance of main effects when significant higher order interactions are present. Modified from Persaud and Williamson 2005. Results from ANOVA (Table 1.1.) suggest that all terms and interactions are significant, but this figure suggests that the main effect of UV is insignificant.

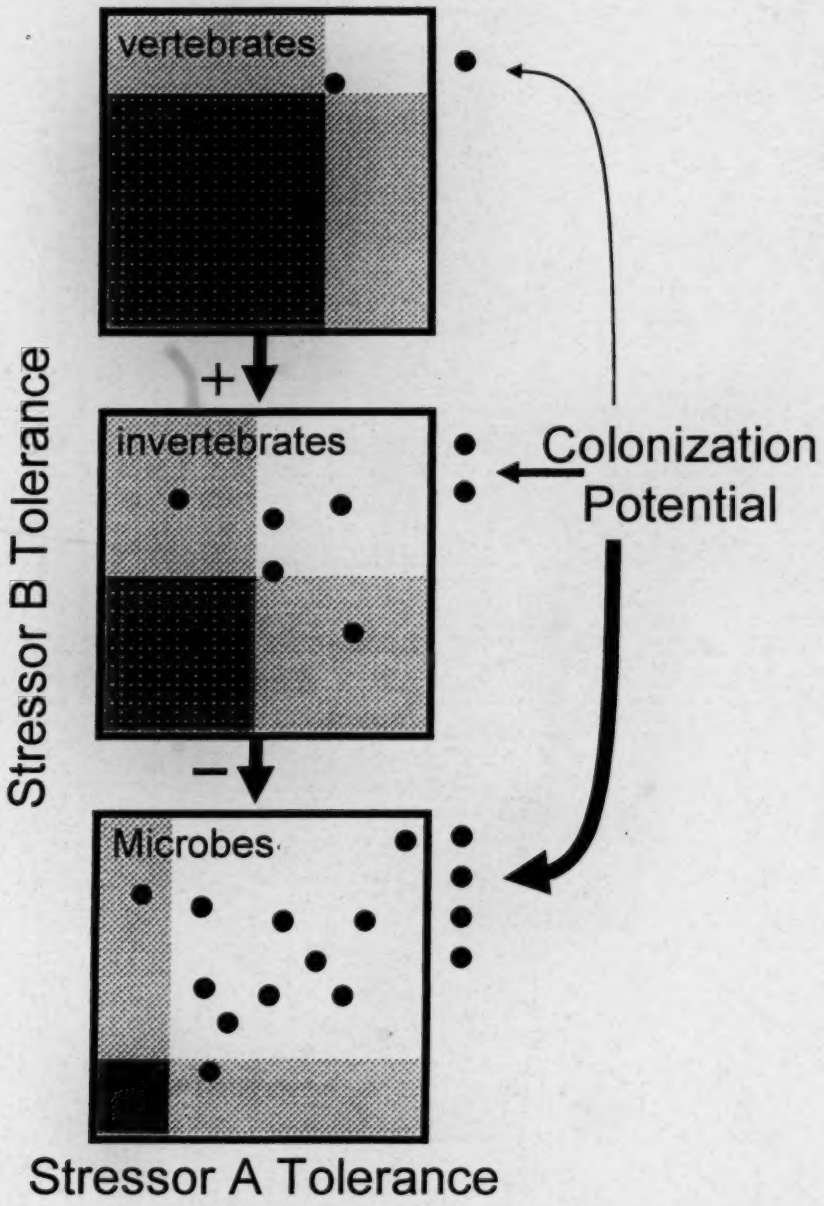

Fig. 1.3. Conceptual diagram demonstrating how trophic levels are predicted to respond to multiple stressors. The tolerance of species or individuals (solid circles) to stressors determines if they would be eliminated (i.e. located within the shaded region) upon exposure to multiple stressors. Lower trophic levels are expected to display antagonistic interactions under multiple stressor scenarios due to high physiological adaptation, greater biodiversity and colonization potential. Conversely, higher trophic levels are expected to display synergistic interactions

CHAPTER TWO

NON-ADDITIVE IMPACTS OF MULTIPLE STRESSORS ON CONSUMERS AND PRODUCERS

INTRODUCTION

Ecosystems are increasingly subjected to the influences of several anthropogenic stressors that define global change (Sala et al. 2001). Stressors typically do not operate independently, but interact to produce non-additive effects that are unpredictable, based upon the knowledge of single stressor studies (Folt et al. 1999). Interactions between stressors have been known to cause "ecological surprises" (*sensu* Paine et al. 1998), dramatically altering the stability of ecosystems. For example, acid deposition synergistically amplifies the sensitivity of boreal lakes to extreme climatic events that involve increased heating events and variation in precipitation (e.g. Yan et al. 1996; Gunn 2002). The uncertainty surrounding the intensity and ubiquity of interactions is a major obstacle of predicting the consequences of global change (Sala et al. 2001).

The ecological mechanisms that determine the impacts of multiple stressors are largely unknown. It has been suggested that biota may respond antagonistically (i.e. be less impacted than predicted), due to stress-induced tolerances protecting individuals to a suite of other stressors (Vinebrooke et al. 2004). For example, warmer temperatures enhance photo-enzymatic repair, increasing the ultraviolet radiation (UV) tolerance of some zooplankton species (Williamson et al. 2002) and acid-tolerant zooplankton have demonstrated higher resistance to contaminants (Moore and Folt 1993). Conversely, stressor combinations could cause synergistic responses (i.e. greater effects than expected), as evidenced by many species exhibiting ecological tradeoffs, where tolerance to one stressor comes at a cost to another (Kneitel and Chase, 2004). For example, the negative effects of a pesticide on *D.* pulex were more pronounced under low oxygen (Hanazato and Dodson 1995). Similarly, the

morphological defence strategy of *Daphnia pulex* against predatory *Chaoborus* was suppressed by high temperatures (Dodson and Wagner 1996).

Boreal aquatic systems are currently subject to the multiple stressor scenarios of warmer temperatures, increased frequency of droughts and widespread acidification (Schindler et al. 1996). Dissolved organic carbon (DOC) plays a critical role in aquatic systems, limiting the penetration of light, ultraviolet (UV) and thermal radiation (Williamson et al. 1999). Temperature, drought and acidification each have a common impact on aquatic systems by reducing DOC. Warmer temperatures and decreased runoff from terrestrial systems have resulted in substantial losses of DOC (Schindler et al. 1997; Snucins and Gunn 2000). Photodegredation of DOC is enhanced under acidic conditions (Wu et al. 2005). Additionally, droughts in areas recovering from acid deposition can cause rapid and severe reductions of pH and DOC (Yan et al. 1996). Systems with low DOC concentrations are particularly threatened, due to the exponential relationship between DOC and UVR penetration (Schindler et al. 1996). Thus warming, drought and acidification each reduce DOC, allowing deeper penetration of light, UV and thermal radiation. In other words, interactions between multiple stressors causing moderate losses of DOC could have large impacts by degrading the abiotic environment of lakes.

Little is known about how aquatic communities will respond to multiple stressors, and in particular, the combined stresses of warming, UV and acidification. Single stressor studies have typically demonstrated negative impacts to consumers, but beneficial responses to some producers for temperature (Beisner et al. 1997; Strecker et al. 2004), UV (Bothwell et al. 1994) and pH (Vinebrooke et al. 2003). Additionally, recent small-scale mesocosm studies have established that interactionsbetween temperature and UV affect zooplankton (Persaud and Williamson 2005) and phytoplankton (Doyle et al. 2005) communities. I hypothesized that zooplankton and phytoplankton community composition would be moderately influenced by single stressors, but increasingly (i.e. interactively) altered by treatment combinations. I also

hypothesized that the effects would be more pronounced on zooplankton then on phytoplankton communities. Lastly, I expected that experimental warming, acidification and drought would produce a community composition similar to what was present in Lake 302 South (Lake 302S) during the late 1980's, as during this time the lake experienced these stressors in combination (see methods).

METHODS

Site History

A 50-d experiment was conducted in Lake 302S at the Experimental Lakes Area in northwestern Ontario (49°40'N, 94°45'W). Lake 302S received experimental additions of sulphuric acid from 1982-1990, lowering pH values to 4.5. Chemical and biological recovery has occurred gradually since 1990 with pre-acidification pH being achieved in 2003. Details of the acidification and subsequent recovery can be found elsewhere (Vinebrooke et al. 2003 and sources within). Warmer and drier conditions prevailed during the mid-to-late 1980's, increasing water temperatures and transparency (Findlay et al. 2001). The regional warming trend coincided with the climax of the acidification experiment, exposing biota to the 'triple whammy' situation of increased water temperature, clarity and acidity (Schindler et al. 1996; Christensen et al. 2006). Zooplankton communities at this time were dominated by *Bosmina longirostris*, *Eubosmina tubicen*, *Daphnia catawba*, *Mesocyclops edax* and *Orthocyclops modestus* while phytoplankton were dominated by *Gymnodinium*, *Peridinium*, and *Cryptomonas spp.* (Vinebrooke et al. 2003) and the metaphytic species *Mougeotia* (Turner et al. 1987).

Experimental design

To test the impacts of multiple stressors on planktonic communities, I performed a 3-factor experiment that reduced DOC (5 vs. 3 mg/l), and pH (6.7 vs. 5.0), and increased temperature (control of 918 degree days vs. increase of 32 degree days) in Lake 302S. Factorial treatment combinations were replicated in

triplicate, and randomly assigned among 24 mesocosms. Mesocosms consisted of rectangular polyethylene bags (1500-L capacity), covered by UV-transparent Plexiglass (Curry Industries, Winnipeg MB). Bags were filled with 64-µm sieved lake water, and suspended from a floating frame. To remove DOC, sieved lake water was passed through a makeshift filter containing activated charcoal (Dynamic Aqua Supplies, Surrey BC). Lab-based tests determined that activated charcoal successfully reduced both colour and DOC from water (*personal observations*). Bags were gradually acidified using sulphuric acid over a 3 day period. Heat capture and retention was achieved by insulating mesocosm walls and using a modified greenhouse canopy design (Strecker et al. 2004). Plexiglass canopies were sealed on top of warmed mesocosms using weather-stripping, while control mesocosms had canopies suspended to allow for convective heat loss. Additional heating was achieved by adding 20-L carboys filled with heated water at 3-d intervals. Temperature was recorded at 1-hr intervals from the midpoint of each enclosure using StowAway® TidbiT® thermal probes (Onset Computer, Pocasset, Massachusetts, USA).

To initiate the experiment, zooplankton and phytoplankton communities were collected by vertical hauls from the deepest location of the lake with a 64-µm Wisconsin net, and added to the bags to obtain densities comparable to the lake. To compensate for the loss of nannoplankton during the DOC-removal process ,vertical hauls with a 10-µm Wisconsin net were added to achieve an initial chlorophyll concentration as found in Lake 302S at the start of the experiment. Water chemistry, phytoplankton, and zooplankton were sampled on Day 0 (July 9), 10, 30 and 50 (Aug 28) using an integrated sampler after bags were thoroughly mixed.

Water chemistry samples were analyzed according to ELA procedures (Stainton et al. 1977). Unfiltered samples were fixed with Lugol's solution for phytoplankton enumeration. Phytoplankton were enumerated using sedimentation chambers and an inverted Leica DM-IRB microscope. Large celled, filamentous and colonial algae were enumerated at 100x, scanning half of

the settling chamber to increase the accuracy for large cells. All other cells were counted at 400x with a minimum of 300 cells and 30 fields of view. Identifications were made based the keys of Prescott (1982). Cell dimensions were measured using a digital imaging system and Openlab™ v. 4.0.1 (Improvision 2002) software, and converted to biovolumes by comparing cell morphologies to geometric shapes of known volumes (Hillebrand et al. 1999). Biovolumes were converted to biomass by assuming a specific gravity of one. Zooplankton were concentrated from 16-L, on to a 73-µm sieve and preserved with 95% ethanol (70% final concentration). Entire zooplankton samples were enumerated using a Leica MZ9.5 steriomicroscope. Identifications were based upon the keys of Edmondson (1959). Biomass of crustacean and rotifer zooplankton were estimated by using length-weight regressions and genus-specific geometrical formulae respectively (McCauley 1984). Zooplankton length and dimensions were also measured using Openlab™.

Statistical Analysis

Repeated measures multivariate analysis of variance (RM-MANOVA) were performed using SPSS v.13.0 (SPSS Inc. 2004) to test for the time dependant effects of warming, acidification and DOC removal, and the four potential interaction terms on zooplankton and phytoplankton community composition. Data were $\log_{10}$ transformed prior to analysis to improve homogeneity of variances. After detection of significant RM-MANOVA's, univariate repeated measure analysis of variance (RM-ANOVA) were run on the most abundant species (according to biomass) to determine species-specific responses to interactive and main effects. Detection of significant interactive effects precluded the evaluation of main effects and lower-order interactions (Zar 1999). To counter this dilemma, 'protected ANOVAs' were run to determine the significance of main effects and two-way interactions (see Christensen et al. 2006 for details).

RESULTS

Abiotic conditions

The experimental warming treatment increased the average ambient temperature of 20.86 °C by 0.7 °C over the entire experiment (Fig. 2.1). Water temperatures were not influenced by DOC, pH, or their interactions (data not shown). DOC removal significantly increased the UVB penetration depth by ~0.5 m in the drought-simulated mesocosm (time x DOC; $F_{2,8} = 12.06$, $p = 0.037$) (Fig. 2.2). The combination of DOC removal and acidification had the highest UVB penetration depth, but neither pH (time x pH; $F_{2,8} = 0.74$, $p = 0.546$) nor their interaction were significant (time x DOC x pH; $F_{2,16} = 3.64$, $p = 0.082$) (Fig 2.2). The increase of UV penetration was extensive considering that DOC was only reduced to 3 mg/L, indicating that large amounts of allochthonous coloured DOC was removed, not colourless autochthonous carbon.

Zooplankton community

The response of zooplankton communities can be simplified by examining the response of taxonomic/functional groups. Cladocerans represent large filter-feeding herbivores; copepods comprise both raptorial-feeding (cyclopoids) and dual-mode raptorial-filtering (calanoids) omnivores, while most rotifers (except predacious *Asplanchna* and *Synchaeta*) are small, relatively inefficient filter feeders. Species within these categorizations can have remarkably different responses to abiotic conditions, predators and prey (Fischer et al. 2001; Vinebrooke et al. 2003); however comparisons at the functional level may indicate that certain feeding strategies are more beneficial then others; allowing for broad-based comparisons to other systems that have different species, but similar functional groups. Our experiment was initially comprised mostly of copepods, but cladocerans increasingly dominated as the experiment progressed (Fig. 2.3).

Significant interactions among the stressors, rather than individual main effects, defined their net impact on the three zooplankton groups (Table 2.1; Fig

2.3). Synergistic interactions between acidification, warming, and reduced DOC levels amplified the total abundance of cladocerans. Similarly, a significant 3-way synergistic interaction among the stressors stijmulated copepod abundance. In contrast, removal of DOC antagonistically dampened the positive effects of acidification and warming on total rotifer biomass.

Seven cladocerans (*Daphnia catawba, Daphnia retrocurva, Daphnia longiremis, Holopedium gibberum, Chydorus sphaericus, Bosmina longirostris, Eubosmina tubicen*) two copepods (*Diaptomus sp. and Mesocyclops edax*) and one rotifer (*Keratella taurocephala*) were regularly detected in the mesocosms during the experiment. These ten species collectively accounted for 91.2 % of the total zooplankton biomass during the experiment, and were therefore representative of the whole community. A complete listing of observed zooplankton taxa is provided in Appendix A.

Significant interactions among the stressors were also the primary drivers of changes in zooplankton species composition (Table 2.2; Table 2.3; Fig. 2.4). Synergistic interactions among stressors stimulated the abundance of *Daphnia catawba* despite the negative effects of individual stressors (Fig. 2.4a). Similarly, interactions between reduced DOC and warming or acidity synergistically amplified the total biomass of *Holopedium*, while acidity had a direct negative impact (Fig. 2.4b). The positive effects of acidification and low DOC on *Bosmina* were dampened antagonistically by warming (Fig. 2.4c). Warming and reduced DOC also antagonistically suppressed the positive effect of acidification on *Eubosmina* (Fig. 2.4d). Interactions between acidity and the other stressors exerted significant antagonistic impacts on *Eubosmina*. Unresponsive to all single stressors, *Diaptomus* decreased under temperature x pH, and increased under temperature x DOC and the triple-stressor combination (Fig. 2.4e). The combined effect of the three stressors synergistically amplified the abundance of *Mesocyclops* (Fig. 2.4f). Warming and removal of DOC reduced the positive effect of acidification on *Keratella* biomass (Fig. 2.4 g). All three stressors

synergistically amplified the total biomass of *Chydorus* (Fig. 2.4 h). Drought conditions were basically the only treatment in which *Daphnia longiremis* were present, making statistics problematic due to the large number of zeros in the dataset (Fig. 2.4 i). Each of the main effects hindered *Daphnia retrocurva*, but the species had a large response of the temp x pH treatment, indicative of an antagonistic interaction.

Phytoplankton community

At the functional level, phytoplankton taxa can be categorized according to size. Due to morphological constraints, filter-feeding zooplankton cannot typically consume algae > 35 µm making a simple distinction between edible and non-edible species (Sommer et al. 2001). The size-class of phytoplankton communities can have important bottom-up effects in determining abundance and composition of zooplankton and vice versa (Sommer et al. 2001).

The abundance of 'edible' algae generally declined over time and was highest in treatments receiving acid, and acid combined with warmer temperature (Table 2.4; Fig. 2.5); however these two treatments also had the highest amounts of total phytoplankton. The percent of edible phytoplankton (data not shown) was not consistent to any treatment or zooplankton community (i.e. the abundance of *Daphnia* nor cladocerans could not explain amounts of edible phytoplankton). The phytoplankton community was dominated by four inedible (*Mougeotia* sp., *Peridinium* spp., *Gymnodinium* sp., *Staurodesmus* spp.) and six edible (small chrysophyte sp., large chrysophyte sp. *Chrysochromulina* sp. *Merismopedia* spp., *Chromulina sp.* and *Mallomonas spp*) taxa, which collectively accounted for 88.0% of the total phytoplankton biomass during the experiment.

Phytoplankton were typically identified to species; however, species of the same genus generally displayed similar trends, and were combined prior to analysis. Identification of the small and large chyrsophyte species was limited to

the lack of taxonomic features. The small chyrsophyte sp. was likely similar to *Chromulina* or *Oochromonas*; however no flagella was observed and thus was categorized as a separate taxa. Similarly, the large chyrsophyte sp. was roughly similar in size and morphology to *Chrysochromulina*; however; no flagella was located. Mesocosms were initially dominated by chrysophytes (*Chrysochromulina*, and two unidentified chrysophyte species) and dinoflagellates (*Gymnodinium* and *Peridinium*), but *Mougeotia* became increasingly abundant over time (Fig. 2.6). A complete listing of observed phytoplankton taxa is located in Appendix B. A complete listing of the biomass in each treatment combination for both phytoplankton and zooplankton communities, in addition to the results of the chemical analyses are provided in a CD in Appendix C

Interactions between warming, reduced DOC, and acididfication significantly altered the phytoplankton community composition (Table 2.5; Table 2.6). The three stressors exerted an antagonistically positive effect on *Mougeotia* towards the end of the experiment (Fig. 2.6a). Acidic conditions benefited *Peridinium* but the temperature x DOC interaction caused significant declines (Fig. 2.6b). Similarly, acidification stimulated *Gymnodinium*; however simultaneous exposure to all stressors suppressed this species (Fig. 2.6c). Interaction between warming and reduced DOC antagonistically dampened their individual positive effects on an abundant small chrysophyte (Fig. 2.6d). Warming synergistically amplified the positive effect of acidification on a large unidentified chrysophyte and *Chrysochromulina* (Fig. 2.6e, f). Reduced DOC levels antagonistically suppressed the positive effect of acidification on *Merismopedia* (Fig. 2.6g). Interactions among the three stressors suppressed their individual positive effects on the abundance of *Staurodesmus* (Fig. 2.6h). The direct positive effect of acidification on *Chromulina* was reversed by warming and reduced DOC levels (Fig. 2.6 i). None of the treatment combinations had a significant effect on abundances of *Mallomonas* (Fig. 2.6 j).

DISCUSSION

Temperature, acidification and loss of DOC have each been shown individually to constitute significant stressors to boreal aquatic communities (Holt et al. 2003; Molot et al. 2004; Baulch et al. 2005). However, my study demonstrates the high potential for these three stressors to cause non-additive effects among biota; modifying and altering community composition from what would have been predicted from single-stressor studies. I also showed that individual species typically respond in a highly unpredictable (i.e. interactive) fashion when stressors occurred in combination.

Ecological co-tolerances and trade-offs

Generally, taxa exhibited one of two responses: 1) benefit from the effects of a single-stressor, but multiple stressors resulted in detrimental non-additive effects, or 2) were relatively unresponsive to main effects, but synergistically flourished under stressor combinations (Fig. 2.4, 2.6). These responses are termed trade-offs and co-tolerances respectively, and result from the non-additive responses of a species to multiple stressors. Species exhibiting trade-offs can be thought of as 'specialist' species, possessing specific traits that allow for dominance under certain environmental conditions (i.e. a single stressor) (Kneitel and Chase 2004). However, the impacts of several stressors, each affecting different physiological processes, should exclude specialist species as they would not have tolerances to a wide diversity of stressors. In contrast, co-tolerances are likely found among 'generalist' species, which remain largely unaffected by environmental perturbations, (i.e. 'jack-of-all-trades is the master of none'). Generalist species should dominate frequently (and perhaps multiply) perturbed systems due to the decline of specialist species (Richmond et al. 2005).

Ecological trade-offs were demonstrated by several acid-tolerant species (e.g. *Bosmina*, *Eubosmina*, *Chrysochromulina* and *Gymnodinium*). These species obviously benefited from acidic conditions, but were seemingly intolerant to combinations of acidity and additional stressors. *Bosmina* and *Gymnodinium* in particular are widely regarded as being dominant species under acidic conditions (Findlay et al. 1999; Fischer et al. 2001; Holt et al 2003). *Bosmina* in particular has comprised 99% of zooplankton biomass in some atmospherically acidified lakes (Yan and Strus 1980). However, the experiment predicts that *Bosmina* and *Gymnodinium* do not perform well under both acidic and warmed conditions, which is problematic considering the geographical overlap of these two stressors (Schindler et al. 1996; Gunn 2002). Thus I have demonstrated that several species appear to express ecological trade-offs: they thrive under acidic conditions, but acid tolerance appears to correspond to heightened sensitivity to additional stressors. These species are predicted to dominate acid lakes but are not expected to persist, to the same extent, in systems subject to multiple stressors (Vinebrooke et al. 2004).

In contrast, several species appeared to be co-tolerant, either displaying preference or irrelevance for multiple-stressor situations. For example, *Mesocyclops*, *Daphnia catawba*, *Mougeotia*, and *Merismopedia* showed minimal responses to the main effects, but displayed greater abundances under increasing numbers of stressors. Additionally, *Holopedium*, and the small chrysophyte sp., had significant responses to some of the main effects and interactions; however their responses did not show dramatic benefits or detriments to any treatment combination. In other words, these six species were capable of co-tolerating the integrated impacts of acidification, warming and DOC removal. These species either had stronger affinities for stressful abiotic conditions, or benefited from reduced competition with sensitive species. I would expect that species displaying co-tolerances such as these, would likely become dominant in systems subject to multiple stressors.

Comparison and insight to Lake 302S

Biota in the stressed mesocosms were generally quite similar to what was found in Lake 302S during the height of the acidification experiment in the late 1980's. Zooplankton species such as *Daphnia catawba*, *Eubosmina*, *Bosmina* and *Mesocyclops* are generally considered to be acid-tolerant (Fischer et al. 2001; Holt et al. 2003) and each proliferated in Lake 302S (Vinebrooke et al. 2003) and our experiment. Results from the mesocosm suggest that the later three species are acidophilic, having increased abundances under acidic conditions either due to physiological requirements or reduced competition. However; in the experiment, *Daphnia* was significantly inhibited by acidification (Fig. 2.3a). It has been suggested that increases of *Daphnia catawba* in acidic waters is not simply a direct effect of acidity, but rather the loss of competitors (Fischer et al. 2001) or the reduction of fish predation (Tessier 1986; Vinebrooke et al. 2003). The experiment suggests that *Daphnia catawba* requires warmer temperatures in combination with other stressors in order to thrive, a situation that occurred in Lake 302S (Schindler et al. 1996; Christensen et al. 2006) and other acidified lakes (Frost et al. 1999; Gunn 2002). Thus *D. catawba* appears not to respond to the direct effects of lower pH, or the indirect loss of competitors and predators, but rather the non-additive effects of increased water clarity and temperature.

Not only were similar species dominant in the stressed mesocosms and Lake 302S, but previous analysis of the Lake 302S data set (see Fig. 4 in Vinebrooke et al. 2003) provides additional evidence that certain zooplankton species can display trade-offs between temperature and pH. For example, ordination analysis revealed that both *Eubosmina* and *Bosmina* increased under acidic conditions, but their abundances were greater under lower temperatures, exactly as results from the mesocosm experiment suggested.

Phytoplankton genera such as *Peridinium*, *Gymnodinium*, and *Mougeotia* dominated acidic Lake 302S and our stressed mesocosms. Interestingly, in our

experiment, *Mougeotia* did not increase when exposed to acid alone, but did so only with interactions that included pH. Others have shown that *Mougeotia* has a pH optimum of 8, but thrives in acidic lakes due to being a superior competitor and loss of grazers (Graham et al. 1996). In our study, *Mougeotia* required the triple-stressor combination of warming, pH and reduced DOC before significantly increasing, similar to the environmental conditions of Lake 302S. Thus *Mougeotia* may not be associated with pH *per se*, but rather other environmental stressors that are driven by interactions with acidification, such as increases in water temperature and clarity (Schindler et al. 1996; Frost et al. 1999). Support for co-tolerances and trade-offs among phytoplankton species from the Lake 302S data set (see Fig. 1 in Vinebrooke et al. 2003) was not as prominent and partially obscured by different species assemblages. However, *Peridinium* which in the experiment displayed a co-tolerance between temperature and pH (performed well under acidified conditions regardless of temperature) was clearly associated with warm acidic conditions in Lake 302S.

The large number of significant interaction terms suggests that both zooplankton and phytoplankton communities are highly responsive to the interactions among temperature, DOC and pH. Communities experiencing multiple interacting stressors were significantly different in composition, compared to those exposed to a single stressor, or what was expected assuming that stressors responded in additive fashions. Results from the mesocosm experiment in conjunction with previous results, confirm that biota in Lake 302S respond not only to the direct effects of pH, but are additionally being strongly influenced by interactions among multiple stressors.

Co-tolerances and trade-offs appeared to be evenly distributed between and within trophic and functional groupings. For example, dominant zooplankton taxa were just as likely to tolerate multiple stressor combinations as phytoplankton, refuting our hypothesis that higher trophic levels would be more sensitive. Similarly, the response to interactive effects was independent of

feeding strategy and size for zooplankton and phytoplankton respectively. Lastly, species known to be 'stress tolerant' did not display a greater number of co-tolerances rather then trade-offs, suggesting that 'ecological surprises' such as the loss/reduction of dominant taxa can be expected to occur under multiple stressor scenarios. These results suggest that interactive effects are seemingly random, and that prior knowledge of a species function, ecology and trophic position may have little bearing on its response to multiple stressors.

Limitations of Mesocosm Results

My mesocosm study did not result in abiotic interactions as expected. Increased water clarity as a result of less DOC and lower pH did not cause warmer water temperatures. Similarly, DOC and pH treatments both increased UVR penetration, but the combination of the two resulted in additive effects. Our results suggest that abiotic interactions do not occur over short-time scales within mesocosm studies. The lack of interactions does not suggest that the effects of multiple stressors are negligible to the abiotic environment. For example, additive responses between DOC and pH still created the most detrimental UV environment. Additionally, larger, ecosystem-scale processes that could not have occurred in the mesocosms could result in non-additive effects (e.g. Yan et al. 1996).

It is impossible to predict the exact cause of species-specific responses to each treatment combination. For example, the low abundance of *Bosmina* under the triple stressor condition (Fig. 2.3d), could have been caused by a negative response to the interactive effect of temperature, or being out-competed by the large population of *Daphnia*, or the increase of an inedible algal species such as *Mougeotia*. Lab-based monoculture experiments could be used to help identify individual species' tolerances to multiple stressors. However, I hypothesize that interactions between multiple stressors did not create abiotic conditions that are overly hostile to most species. Instead I propose that these new conditions were

beneficial to competing species, and thus competition for resources explains the abundance of species in each treatment combination.

CONCLUSION

It is widely known that climate change and acidification have large potentials to reduce DOC and deteriorate the abiotic conditions of lakes (Schindler et al. 1996). However, it is critical to understand how biota respond to multiple stressors, as their communities could be substantially more fragile or resilient then expected. My results demonstrate that individual species respond to multiple stressors in highly interactive (non-additive) fashions, displaying a wide range of co-tolerances and trade-offs, making forecasts of biological responses to future global change difficult. Maintenance of ecosystem function appears to be highly dependant on the prevalence of co-tolerances among multiple stressors. In contrast, a prevalence of trade-offs would suggest that 'stress tolerant' species might be highly susceptible to additional stressors, thus presenting a potential disruption to ecosystem function. Results from the mesocosm experiment suggest that co-tolerant species were able to compensate for the diminished abundance of those with trade-offs. However, the strong trade-offs between temperature and pH among widely regarded stress tolerant species (*Bosmina* and *Gymnodinium*) is certainly a cause for concern. Clearly, further experimental studies are required to determine the role of interactions at population, community and ecosystem scales. Future predictions of global change will need to account for the highly interactive nature of anthropogenic stressors (Sala et al. 2000).

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Table 2.1 RM-ANOVA testing for the effects of temperature (Temp), DOC, pH and associated interactions on log-transformed cladoceran, copepod and rotifer biomass. Significant p-values appear in bold.

Cladoceran

Source	df	*F*	*P*
Temp	1	1.527	0.234
DOC	1	17.333	**0.001**
pH	1	19.085	**< 0.001**
Temp x DOC	1	26.758	**< 0.001**
Temp x pH	1	32.669	**< 0.001**
DOC x pH	1	19.007	**< 0.001**
Temp x DOC x pH	1	1.313	0.269

Copepod

Source	df	*F*	*P*
Temp	1	8.776	**0.009**
DOC	1	77.869	**< 0.001**
pH	1	1.3	0.271
Temp x DOC	1	33.091	**< 0.001**
Temp x pH	1	0.564	0.463
DOC x pH	1	3.554	0.078
Temp x DOC x pH	1	9.336	**0.008**

Rotifer

Source	df	*F*	*P*
Temp	1	9.026	**0.008**
DOC	1	43.643	**< 0.001**
pH	1	250.87	**< 0.001**
Temp x DOC	1	11.29	**0.004**
Temp x pH	1	0.179	0.678
DOC x pH	1	7.813	**0.013**
Temp x DOC x pH	1	0.946	0.345

Table 2.2. Repeated-measures MANOVA testing for the time-dependant effects of temperature (Temp), DOC, pH and associated interactions on log-transformed zooplankton biomass for the most abundant six species.

Source	df	*F*	*P*
Time	12	544.42	**< 0.001**
Time x Temp	12	23.02	**0.001**
Time x DOC	12	27.96	**0.001**
Time x pH	12	63.90	**< 0.001**
Time x Temp x DOC	12	23.40	**0.001**
Time x Temp x pH	12	12.92	**0.003**
Time x DOC x pH	12	42.55	**< 0.001**
Time x Temp x DOC x pH	12	24.32	**0.001**

Table 2.3. Zooplankton species response to treatment combinations (Values are p-values from 'protected' ANOVAs).

Significant p-values appear in bold. n/a denotes samples were no individuals were observed, and thus statistics could not be performed.

Between sujects Source	*Daphnia catawba*	*Holopedium gibberium*	*Eubosmina tubicen*	*Bosmina logirostris*	*Diaptomus sp.*	*Meso-cyclops edax*	*Keratella taurcephala*	*Daphnia longiremis*	*Chydorus sphericus*	*Daphnia retrocurva*
Temp	**0.004**	0.062	**0.042**	0.792	0.310	0.218	**0.003**	n/a	**< 0.001**	0.063
DOC	**< 0.001**	0.078	0.971	**0.002**	0.117	0.815	**0.001**	**< 0.001**	**0.006**	**0.007**
pH	**< 0.001**	**0.033**	**0.008**	**0.003**	0.838	**0.001**	**0.005**	n/a	0.298	**0.028**
Temp x DOC	**< 0.001**	**0.007**	0.756	**< 0.001**	**0.023**	0.306	**< 0.001**	**< 0.001**	**< 0.001**	0.083
Temp x pH	**< 0.001**	0.339	**0.040**	**0.003**	**0.025**	0.320	0.135	n/a	**< 0.001**	**< 0.001**
DOC x pH	**< 0.001**	**0.004**	**0.003**	**0.002**	0.637	**0.042**	0.270	**< 0.001**	**0.002**	**0.007**
Temp x DOC x pH	**< 0.001**	0.385	**0.018**	**0.009**	**0.014**	**0.004**	0.348	**< 0.001**	**< 0.001**	**< 0.001**

Within subjects (Multivariate) - Time and time x treatment effects

Source	*Daphnia catawba*	*Holopedium gibberium*	*Eubosmina tubicen*	*Bosmina logirostris*	*Diaptomus sp.*	*Meso-cyclops edax*	*Keratella taurcephala*	*Daphnia longiremis*	*Chydorus sphericus*	*Daphnia retrocurva*
Time	**0.028**	0.148	**0.036**	0.179	**0.041**	0.333	0.051	n/a	0.111	0.518
Time x Temp	**0.004**	0.631	0.083	**0.027**	0.614	**0.001**	**0.003**	n/a	**0.001**	0.103
Time x DOC	**< 0.001**	0.457	0.363	**0.002**	**0.014**	**0.352**	0.007	**< 0.001**	**0.029**	0.262
Time x pH	**0.010**	**0.001**	**0.014**	**0.010**	**0.026**	0.101	**0.006**	n/a	0.250	0.149
Time x Temp x DOC	**< 0.001**	0.095	0.110	**0.021**	0.068	0.505	**< 0.001**	**< 0.001**	**0.002**	0.498
Time x Temp x pH	**< 0.001**	0.253	**0.015**	**0.004**	0.262	**0.002**	**0.003**	n/a	**< 0.001**	0.080
Time x DOC x pH	**0.021**	**0.002**	**0.002**	**0.003**	**0.007**	**0.010**	**< 0.001**	**< 0.001**	**0.002**	0.986
Time x Temp x DOC x pH	**< 0.001**	0.211	0.559	**0.001**	**0.042**	0.622	**< 0.001**	**< 0.001**	**< 0.001**	0.071

Table 2.4 RM-ANOVA testing for the effects of temperature (Temp), DOC, pH and associated interactions on log-transformed edible and inedible phytoplankton biomass. Significant p-values appear in bold.

Inedible

Source	df	*F*	*P*
Temp	1	12.019	**0.003**
DOC	1	10.934	**0.004**
pH	1	2.495	0.134
Temp x DOC	1	0.003	0.958
Temp x pH	1	0.409	0.531
DOC x pH	1	0.03	0.864
Temp x DOC x pH	1	16.998	**0.001**

Edible

Source	df	*F*	*P*
Temp	1	1.605	0.223
DOC	1	51.783	**< 0.001**
pH	1	128.117	**< 0.001**
Temp x DOC	1	0.47	0.503
Temp x pH	1	0.374	0.55
DOC x pH	1	17.203	**0.001**
Temp x DOC x pH	1	2.766	0.116

Table 2.5. Repeated-measures MANOVA testing for the time-dependant effects of temperature (Temp), DOC, pH and associated interactions on log-transformed phytoplankton biomass for the most abundant eight species.

Source	df	*F*	*P*
Time	16	2631.19	**< 0.001**
Time x Temp	16	66.29	0.096
Time x DOC	16	412.62	**0.039**
Time x pH	16	1259.83	**0.022**
Time x Temp x DOC	16	1865.85	**0.018**
Time x Temp x pH	16	212.06	**0.054**
Time x DOC x pH	16	47.60	0.133
Time x Temp x DOC x pH	16	57.54	0.103

Table 2.6. Phytoplankton species response to treatment combinations (Values are p-values from 'protected' ANOVAs). Significant p-values appear in bold.

Between sujects

Source	*Mougeotia spp.*	*Peridinium sp.*	*Gymnodinium sp*	small chrysophyte sp	large chrysophyte sp.	*Chrysochromilina sp.*	*Merismopedia spp.*	*Staurodesmus spp*	*Chromulina sp.*	*Mallomonas spp.*
Temp	0.180	0.061	0.467	**0.005**	0.643	**0.015**	0.991	0.718	0.175	0.058
DOC	0.417	0.359	0.833	**0.003**	0.076	**0.001**	**0.004**	**0.035**	0.287	0.931
pH	0.929	**0.010**	**0.001**	**< 0.001**	**0.013**	0.093	**< 0.001**	**0.007**	**0.022**	0.736
Temp x DOC	0.489	**0.033**	0.770	**0.021**	0.846	**0.002**	**0.017**	**0.022**	0.093	0.124
Temp x pH	0.067	0.146	**0.028**	0.087	**0.002**	**< 0.001**	0.076	**0.036**	**0.015**	0.661
DOC x pH	0.118	0.098	**0.001**	**< 0.001**	**0.017**	**0.012**	0.087	**0.001**	**0.025**	0.717
Temp x DOC x pH	**0.007**	0.232	**0.038**	0.096	0.137	**< 0.001**	0.407	**0.001**	**0.003**	0.708

Within subjects (Multivariate) - Time and time x treatment effects

Source	*Mougeotia spp*	*Peridinium sp.*	*Gymnodinium sp*	small chrysophyte sp	large chrysophyte sp.	*Chrysochromilina sp.*	*Merismopedia spp*	*Staurodesmus spp*	*Chromulina sp.*	*Mallomonas spp.*
Time	**0.009**	**0.028**	**0.043**	**< 0.001**	**0.002**	**< 0.001**	0.591	**0.050**	0.154	0.172
Time x Temp	**0.008**	**0.031**	0.461	0.727	0.595	0.062	0.674	0.287	0.837	0.061
Time x DOC	0.214	0.505	0.225	0.149	0.507	**0.005**	0.087	**0.002**	**0.027**	**0.008**
Time x pH	0.069	0.132	**0.023**	0.239	**0.029**	**0.043**	**< 0.001**	**0.005**	**0.034**	0.270
Time x Temp x DOC	**0.008**	**0.002**	0.111	0.767	0.102	**0.010**	0.061	**0.010**	0.890	0.207
Time x Temp x pH	**< 0.001**	0.111	0.142	0.629	0.905	**< 0.001**	0.281	0.185	0.219	0.399
Time x DOC x pH	**0.015**	0.144	0.287	0.142	0.079	0.346	**0.028**	**< 0.001**	**0.001**	0.484
Time x Temp x DOC x pH	**< 0.001**	0.694	0.904	0.696	0.227	0.117	0.283	**0.029**	0.691	0.501

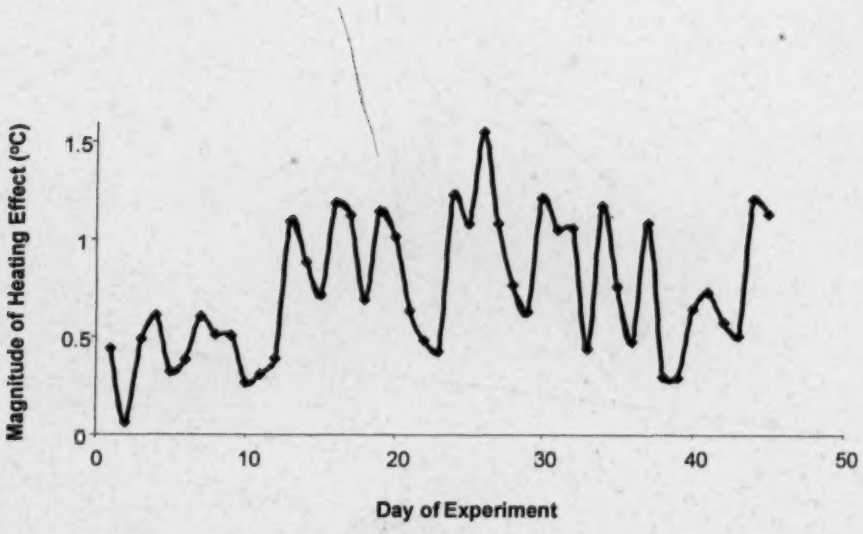

Fig. 2.1. Representative temperature difference between an insulated and control bag from July 9 to August 28. Readings were obtained hourly using data loggers located at the midpoint of each mesocosm. Temperature differences reported here are daily averages.

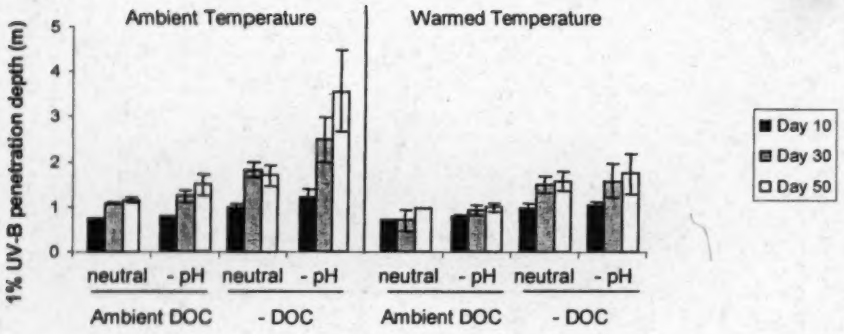

Fig. 2.2. Calculated 1% penetration depth of UVB within the mesocosms, for each treatment combination (n = 3). Error bars indicate standard error.

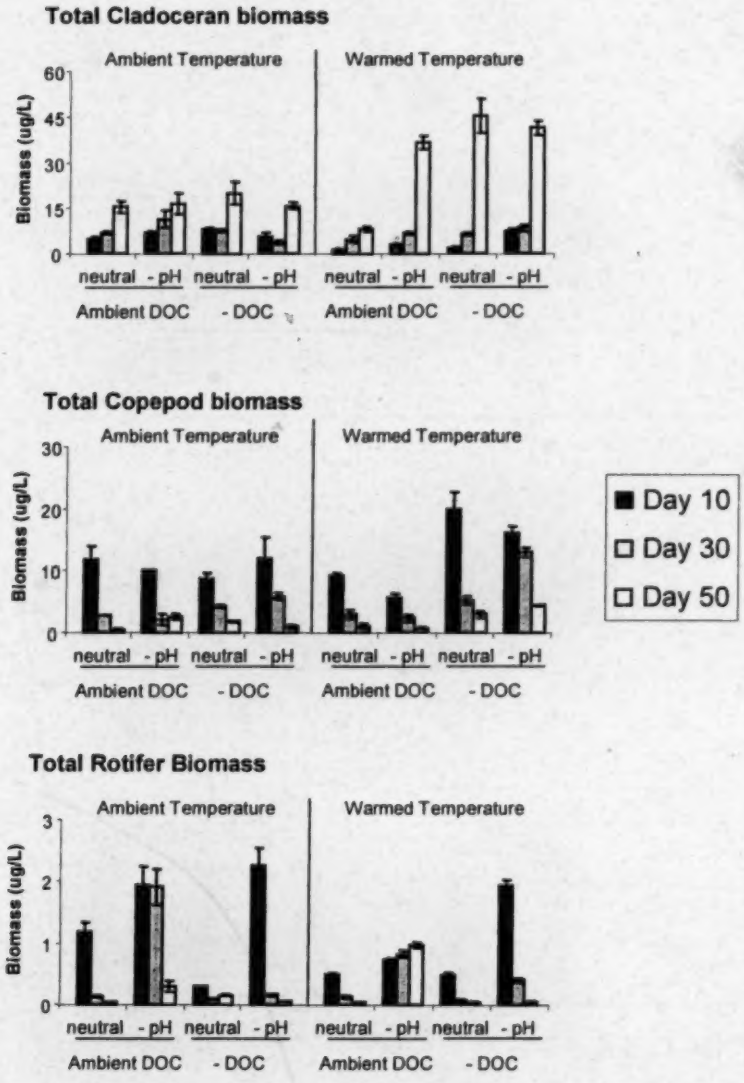

Fig. 2.3. Biomass zooplankton taxonomic/functional groups on each sampling date: a) cladocerans, b) copepods and c) rotifers (n = 3). Error bars indicate standard error.

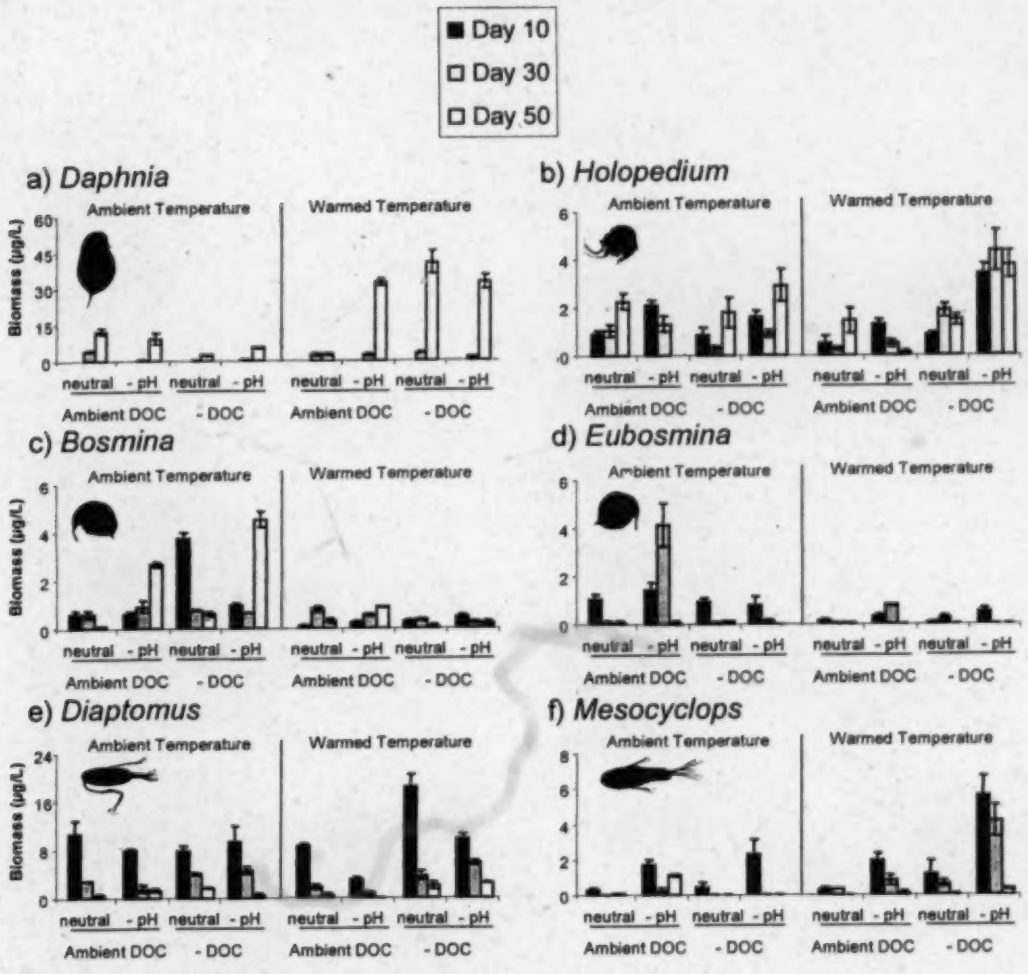

Fig. 2.4. Biomass of major zooplankton species: a) *Daphnia catawba* b) *Holopedium gibberum* c) *Eubosmina tubicen* d) *Bosmina longirostris* e) *Diaptomus sp.* f) *Mesocyclops edax* in response to warming, acidification (- pH) and DOC removal (- DOC) over the duration of the experiment (n = 3). Error bars indicate standard error.

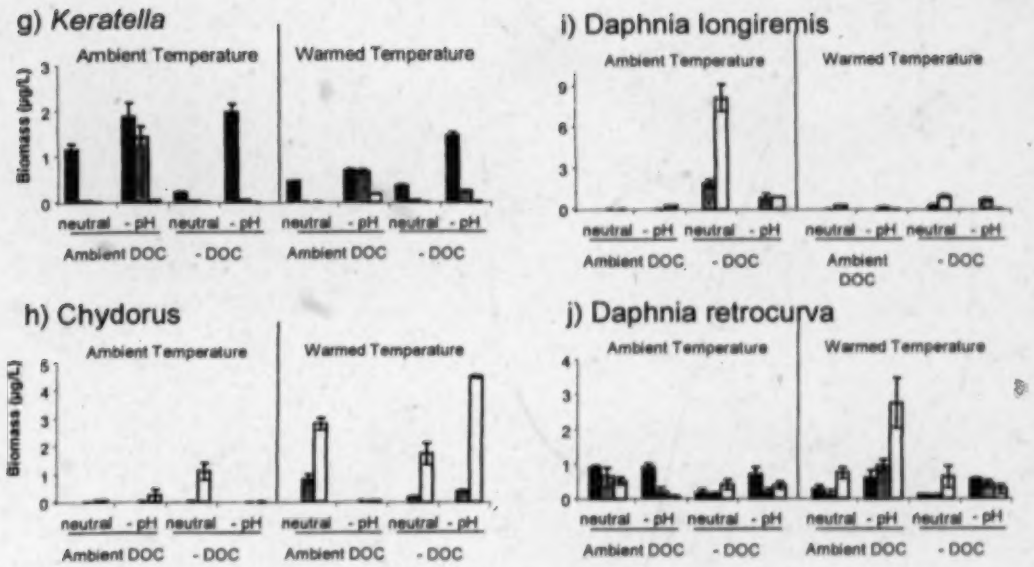

Fig. 2.4b. Biomass of major zooplankton species: **g)** *Keratella taurocephala* **h)** Chydorus sphericus **i)** Daphnia longiremis **j)** Daphnia retrocurva

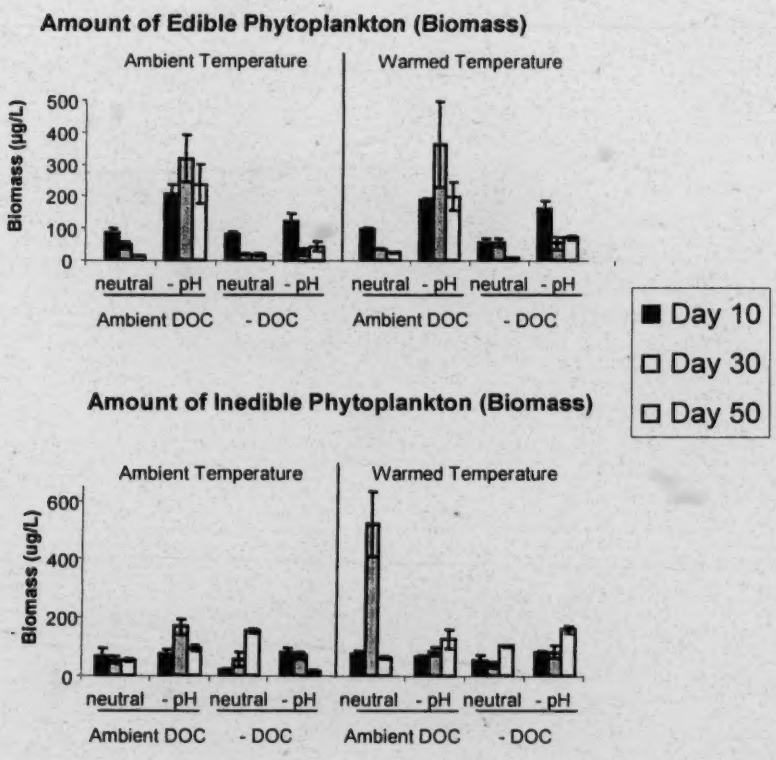

Fig. 2.5. Biomass of edible phytoplankton within mesocosms at each sampling date (n = 3). Error bars indicate standard error.

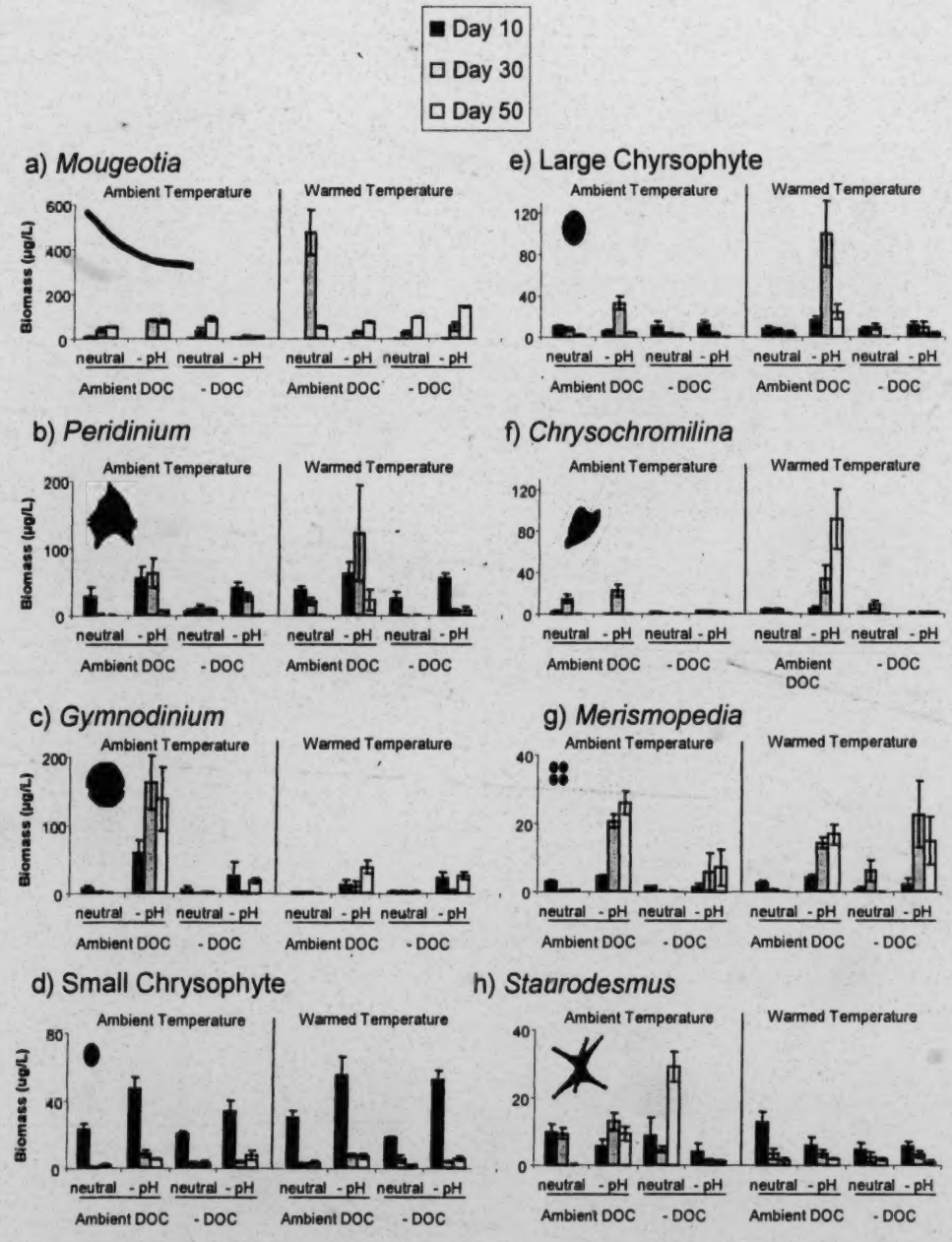

Fig. 2.6. Biomass of major zooplankton species: a) *Mougeotia sp.* b) *Peridinium sp.* c) *Gymnodinium sp.* d) small chrysophyte sp. e) large chrysophyte sp. f) *Chrysochromulina sp.* g) *Merismopedia sp.* h) *Staurodesmus sp.* in response to warming, acidification (- pH) and DOC removal (- DOC) over the duration of the experiment (n = 3). Error bars indicate standard error.

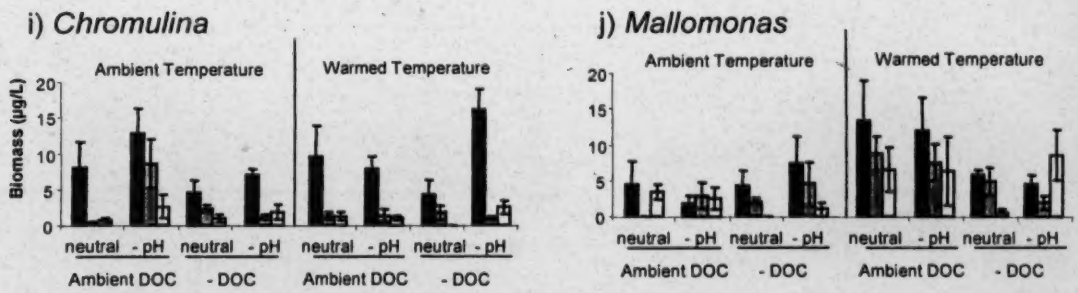

Fig. 2.6b. Biomass of major phytoplankton species: i) *Chromulina* sp. j) *Mallomonas spp.*

CHAPTER THREE

MULTIPLE ANTHROPOGENIC STRESSORS CAUSE ECOLOGICAL SURPRISES IN BOREAL LAKES

INTRODUCTION

Multiple anthropogenic stressors drive the cumulative impacts on global change on biodiversity and ecosystem function (Sala *et al.*, 2000; Steffen *et al.*, 2004). In particular, stressors are expected to exert complex interactive effects given the ubiquity of ecological trade-offs (Kneitel & Chase, 2004), stress-induced tolerances (Blanck 2002), and trophic differences in environmental sensitivity (Vinebrooke *et al.*, 2003; Raffaelli, 2004). Unfortunately, interactions among multiple stressors cannot be easily modelled because they generate net impacts that either exceed (i.e. synergism) or fall below (i.e. antagonism) their expected additive effects (Folt *et al.*, 1999). Our understanding of the ecological effects of global change remains limited by a lack of theory (but see Vinebrooke et al. 2004) and empirical evidence derived from multi-factorial investigations (e.g., Doyle *et al.*, 2005; Henry *et al.*, 2005; Persaud *et al.*, 2005; Preslawski *et al.*, 2005). Consequently, global change is often characterized by unexpected non-additive *ecological surprises* (*sensu* Paine *et al.*, 1998).

Several scenarios demonstrate the high probability of interactions among ecological stressors generating non-additive impacts on aquatic ecosystems. For instance, warming antagonistically reduces UVB effects in certain organisms by enhancing photo-enzymatic repair (Williamson *et al.*, 2002; MacFayden *et al.*, 2004). Conversely, warming may synergistically amplify the adverse effects of UVB on organisms that rely on cold-induced photoprotective pigmentation (Hairston, 1979). Further, acidification may antagonistically suppress biological effects of other stressors by inducing a shift towards smaller body size, which is a common trait of many stress-tolerant organisms (Cattaneo *et al.*, 1998;

Vinebrooke *et al.*, 2004). Also, differential stressor effects on adjacent trophic levels can alter their interactions, thereby further increasing the probability that multiple ecological stressors will have complex and non-additive cumulative impacts. Therefore, a high degree of uncertainty exists in predicting the cumulative impacts of anthropogenic stressors on an ecosystem because stress-induced species tolerances produce antagonistic responses, while ecological trade-offs generate synergy.

Boreal landscapes around the world contain the greatest number of lakes that are susceptible to the interactive effects of variable climate and acid deposition (Schindler 1998). Warmer and drier climatic conditions together with acid rain suppress concentrations of terrestrially-derived, light-attenuating dissolved organic carbon (DOC) in lakes, thereby increasing their sensitivities to warming events (Snucins & Gunn, 2000) and biologically-damaging ultraviolet radiation-B (UVB) (Schindler *et al.*, 1996). Drought-induced declines in DOC also stress northern temperate lake ecosystems because of their heterotrophic dependency on terrestrial subsidies (del Giorgio *et al.*, 1999). Drought also synergistically increases the impacts of acidic sulphur deposition on lakes by causing pronounced re-acidification events and loss of UV-attenuating DOC (Yan *et al.*, 1996; Clark *et al.*, 2005). However, it is unknown whether these well-documented interactions among abiotic stressors cause simple additive or unpredictable complex non-additive impacts on ecosystem functioning.

I hypothesized that interactions among warmer temperatures, drought and acidification, rather than the sum of their individual effects, determine the cumulative impact of global change on planktonic consumers and producers in remote boreal lakes. I also expected that interactions among the three stressors would exert contrasting effects on zooplankton and phytoplankton. These ecological stressors should exert a synergistic impact on consumers while affecting producers in a less pronounced, antagonistic manner because physiological acclimation and adaptation to environmental change decline with

increasing trophic status (Vinebrooke *et al.*, 2003; Raffaelli, 2004). For example, single-stressor studies have shown detrimental impacts of consumers and beneficial responses to producers for increased temperatures (Beisner *et al.*, 1997; Petchey *et al.*, 1999; Strecker *et al.*, 2004), UV (Bothwell *et al.*, 1994), and acidity (Vinebrooke *et al.*, 2003).

MATERIALS AND METHODS

Study area

My investigation of the ecological impacts of anthropogenic stressors focused on Lake 302 South (49°40'N, 94°45'W) at the Experimental Lakes Area (ELA) in northwestern Ontario, Canada. Lake 302S was the site of a whole-lake experiment from 1980 to 1990 when additions of sulphuric acid lowered its pH from 6.8 to 4.5 and reduced DOC from 6 to 1.5 mg/L (Fig. 3.1) Chemical and biological recovery occurred during the 1990s (Findlay *et al.*, 1999) with pH returning to pre-acidification levels by 2003. In addition, climate warming during the 1980s resulted in warmer and drier conditions in the ELA, which further decreased DOC and increased underwater warming and penetration of UVB (Schindler *et al.*, 1996) (Fig. 3.1). Thus, this ecosystem-level investigation simulated the effects of acidification, drought, and climate warming, which similarly affect a wide range of other experimentally (Frost *et al.*, 1999) and atmospherically acidified lakes (Arnott *et al.*, 2001).

Biomonitoring of Lake 302S

Lake 302S was sampled on a bi-weekly basis during the ice-free season (May - October) at a mid-lake sampling station using standard ELA collection protocols (see Findlay *et al.*, 1999 and Vinebrooke *et al.*, 2003 for details). For the purposes of this paper, we examined the abiotic variables of temperature, DOC and pH, and both planktonic consumer and producer biomass. To account for the movement of some biota throughout the water column, we used the lake's

heat content as an integrated measurement of lake temperature. Increases in heat content corresponded to higher air temperatures of 1-2°C, and increased hypolimnetic warming of ~ 2°C (Findlay *et al.*, 2001; data not shown). Each variable was calculated as an annual average.

Mesocosm experiment

A 3-factor experimental design consisting of two treatment levels of acidification (pH 6.7 vs. 5.0), a drought-induced decline in DOC (5 vs. 3 mg/L), and warming (control vs. increase of 32 degree days) was performed in triplicate, and randomly assigned among 24 mesocosms (1500-L capacity). All polyethylene-lined mesocosms were suspended from a floating wooden frame in Lake 302S, and filled with 64-μm sieved lake water on July 9, 2004. Sulphuric acid was gradually added over a 3-d period to acidify 12 mesocosms. Warming was achieved by trapping solar heat using UV-B transparent greenhouse canopies (Strecker et al. 2004), which were positioned on top of 12 double-walled insulated mesocosms. Canopies were sealed on top of warmed mesocosms using weather-stripping, and suspended 2-cm above the other 12 non-insulated units to allow for convective heat loss. Supplementary warming was performed at regular 3-d intervals using 20-L water carboys filled with heated water from a propane water tank positioned on the shoreline. Drought was simulated in mesocosms by diluting with lake water that had been passed through a polyethylene bucket containing 75 kg of activated charcoal, which removed 40% of the DOC and doubled the penetration of UVB from 0.75 to 1-5-m depth without significantly affecting low ambient concentrations of total dissolved phosphorus (< 10 $\mu g\ L^{-1}$ in all mesocosms).

Mesocosms were then inoculated with plankton collected from mid-lake vertical plankton hauls to ensure that all contained equal initial densities that were representative of lake conditions. Abiotic and biotic response variables were sampled on Day 0, 10, 30 and 50. Inverted light microscopy was used to

enumerate phytoplankton, and total biomass calculated by converting geometrically derived taxonomic volumes using a specific gravity of one. Zooplankton taxa were counted using a stereomicroscope, and their biomass calculated using length-weight regressions (McCauley 1984).

Statistical analyses

Backward stepwise regression using temperature (heat content), DOC, and pH was performed to identify significant environmental predictors of log-transformed annual total planktonic consumer and producer biomass from the 23-yr experiment in Lake 302S. An alpha value of 0.05 was used as the criterion for removing variables from the model. To determine the role of interactions among multiple stressors and the relative utility of an additive versus non-additive approach, a subsequent regression analysis involving both individual predictors and their interactions (i.e. temperature x DOC, temperature x pH, DOC x pH, and temperature x DOC x pH) was performed, and the two models were compared using adjusted R^2 values to determine the most parsimonious model. As expected, interaction terms were highly correlated with single predictors. To reduce co-linearity, we rescaled the independent variables by centering (i.e. subtracting the mean from each value) (Aiken and West 1991). Tolerance values and Durbin-Watson statistics were subsequently used to test for co-linearity and auto-correlation, respectively.

Repeated-measures analysis of variance (RM-ANOVA) was conducted to test for the interactive effects of warming, drought, and pH on log-transformed total zooplankton and phytoplankton biomass during the mesocosm experiment. Although detection of significant higher order interactions precludes using the same statistical results to examine lower order interactions and main effects (Zar 1999), these must be quantified to determine the nature (i.e. synergy or antagonism) of higher-order interactions. Therefore, significant 3-way interactions were examined using a series of protected (*sensu* Scheiner 2001) lower-order ANOVAs. For example, a significant 3-way interaction was followed

up with ANOVAs using only controls and groups exposed to single stressor to determine the effect of each stressor. The observed combined impact of all three stressors was then compared with their expected net additive effect, which was based on the sum of their individual effects. If the observed combined impact of stressors exceeded their expected additive effect [e.g. ($Stressor_1$ – Control) + ($Stressor_2$ – Control)], then the interaction was defined as being synergistic. In contrast, if the observed impact was less than the additive effect, then the interaction was denoted by antagonism. To illustrate the nature of the interactive impact, I show graphically the difference between the observed [($Stressor_{1 \times 2}$ - Control)] and predicted additive effects, which indicates the direction and magnitude of the interaction.

RESULTS AND DISCUSSION

Interactions among temperature, DOC, and pH were significant predictors of changes in planktonic consumer and producer biomass in Lake 302S (Table 3.1). Inclusion of interaction terms increased the predictability of additive models by 20.4% for consumers, and 14.4% for producers. Partial regression coefficients showed that the interaction between warming and DOC together with higher pH best explained increases in total consumer biomass, particularly during the late 1990s (Fig. 3.1). In contrast, a significant positive temperature-pH interaction term suggested that warming reversed the positive influence of acidification on total producer biomass, which was most evident as phytoplankton was more abundant under acidified conditions during the colder early 1990s than in the warmer late 1980s (Fig. 3.1). Although these results revealed the potential direction of the impacts of interactions among ecological stressors in Lake 302S, they could not be used to identify the nature (i.e. synergy vs. antagonism) of the interactive effects, and therefore, a multi-factorial experiment was warranted.

My mesocosm experiment generated corroborative evidence of interactions among stressors, rather than individual direct effects, determining their net impact on consumer abundance. Specifically, warmer temperatures

stimulated total zooplankton biomass only under conditions of simulated drought and high acidity towards the end of the experiment (Fig. 3.2a; RM-ANOVA, time-temperature-drought-acid interaction, $F_{2,32} = 11.59$, $P < 0.001$). Interactions among the three stressors synergistically amplified total biomass by 130 – 240% above that predicted based on the sum of their individual main effects (i.e. additive model) during the experiment (Fig. 3.3a). Interestingly, these experimental results agreed with the transient increase in zooplankton abundance that was observed in Lake 302S during the advanced stages of acidification, which coincided with warmer and drier conditions in the late 1980s (Fig. 3.1).

The synergistic positive impact of warming, drought-induced UVB exposure, and acidity on consumer biomass was however unexpected based on earlier reports of their individual negative effects on zooplankton (Moore *et al.*, 1996; Williamson *et al.*, 2002; Vinebrooke *et al.*, 2003; Strecker *et al.*, 2004). The positive response of consumers to these triple stressors was primarily attributable to stimulation of a single stress-tolerant herbivore species (*Daphnia catawba*). *D. catawba* had also become the most abundant planktonic consumer in Lake 302S (Vinebrooke *et al.*, 2003) when lake temperatures, acidity, and risk of UVB-damage were elevated during the late 1980s (Fig. 3.1). Warming enhances photoenzymatic repair in this species (Williamson *et al.*, 2002), offering a potential partial explanation for the positive effect of temperature under simulated drought conditions and elevated exposure to UVB. Further, *D. catawba* is also highly efficient under conditions of severe food-limitation (Tessier, 1986), possibly enabling it to benefit more than its acid-sensitive competitors from the positive effects of warming on feeding rates (Moore *et al.*, 1996) and growth (Gillooly, 2002). Warming can also increase the amplitude of fluctuations in daphnid populations, resulting in dramatic boom-bust cycles (Beisner *et al.*, 1997). Therefore, the pronounced positive triple-stressor effect on consumer biomass in the mesocosms on day 50, and in acidified Lake 302S

during the late 1980s, were likely transient events resulting from stress-induced destabilization of population cycles.

In contrast, stressors exerted a significant antagonistic positive effect on producer biomass during the mesocosm experiment (Fig. 3.2b; RM-ANOVA, temperature-drought-acid interaction, $F_{2,32} = 13.79$, $P = 0.002$). Warming stimulated the interactive effect of drought and acidification on total phytoplankton biomass. However, protected ANOVA revealed that both the 3-way and lower-order interactions involved warming and drought antagonistically suppressing the otherwise positive effect of acidification on producer biomass (Fig. 3.3b). These antagonistic interactions reduced the net impact of the three stressors on phytoplankton abundance by 170 – 750% below their potential additive effect, especially during the latter half of the experiment (Fig. 3.3b).

The positive triple-stressor impact on producer biomass in the mesocosms corresponded with increases in phytoplankton abundance during the acidification of Lake 302S (Fig. 3.1). In both cases, filamentous green algae (*Mougeotia* spp.) proliferated under conditions of increased acidity, warmer lake temperature, and elevated exposure to UVB. I expect that the positive effect of moderate warming on photosynthetic rates (Davison, 1991) stimulated this acidophilic primary producer. Also, drought- and acidification-induced declines in DOC improved light availability in both the mesocosms (data not shown) and Lake 302S (Schindler *et al.*, 1996), likely further stimulating acid-tolerant producers that were co-tolerant of concomitant increases in biologically damaging UVB. However, producers in the mesocosms were suppressed by the combined effect of drought and acidification. This antagonistic impact could reflect photo-inhibition of producers owing to the synergistic positive effect of drought and acidification on light and UVB exposure (Schindler *et al.*, 1996; Yan *et al.*, 1996).

The contrasting synergistic and antagonistic triple-stressor impacts on consumers and producers could not be attributed to the mediating influence of a

trophic interaction. Lack of a significant correlation between total consumer and producer biomass during the experiment ($r^2 = 0.002$ $p = 0.74$) and in Lake 302S ($r^2 = 0.04$, $p = 0.35$) supported other reports that consumer populations are too sparse to exert a significant impact on producers in unproductive ecosystems (Elser & Goldman, 1991). The weak linkage between consumers and producers in the mesocosms was not surprising given the predominance of large inedible algae and small filter-feeding herbivores. Instead, contrasting impacts of ecological stressors on consumers and producers likely reflect the greater sensitivity of higher trophic levels owing to their relative lack of physiological acclimation, genetic adaptation, and species diversity (Vinebrooke *et al.*, 2003, 2004; Raffaeli, 2004).

My results highlight the potential prevalence and magnitude of interactions among anthropogenic stressors, and how the cumulative impact can generate ecological surprises at large and small spatio-temporal scales. Further, my findings reveal that unexpected biological responses in ecosystems that are perturbed by a single major stressor (e.g., acidification) can involve non-additive interactions with other concomitant stressors (Frost *et al.*, 1999; Arnott *et al.*, 2001). Clearly, forecasts of the cumulative impacts of global change on biodiversity and related ecosystem processes will remain highly unreliable without further investigation into the nature of interactions among key anthropogenic stressors in different biomes around the world (Sala *et al.*, 2000; Steffen *et al.*, 2004).

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Table 3.1. Backward stepwise regression results showing significant environmental predictors of mean annual ice-free planktonic consumer and producer biomass over a 23-yr period. Additive models considered only three independent variables (DOC, pH, Temp), while interactive models contained both the single predictors and all of their possible combinations (Temp x DOC, Temp x Acid, DOC x Acid and Temp x DOC x Acid). Temp = Heat content of lake. D-W = Durbin-Watson statistic.

Parameter	Model	Variables	Coefficient	Tolerance	*p*-value	p_{model}	$_{adj}R^2$	D-W
Consumer Biomass	Additive	pH	0.204	1.000	0.001	0.001	0.411	1.301
	Interactive	pH	0.176	0.963	<0.001	<0.001	0.615	2.018
		Temp x DOC	0.147	0.963	0.002			
Producer Biomass	Additive	Temp	-0.120	0.972	0.048	<0.001	0.545	1.382
		pH	-0.245	0.972	0.001			
	Interactive	pH	-0.249	0.982	<0.001	<0.001	0.689	1.728
		Temp x pH	0.279	0.982	0.001			

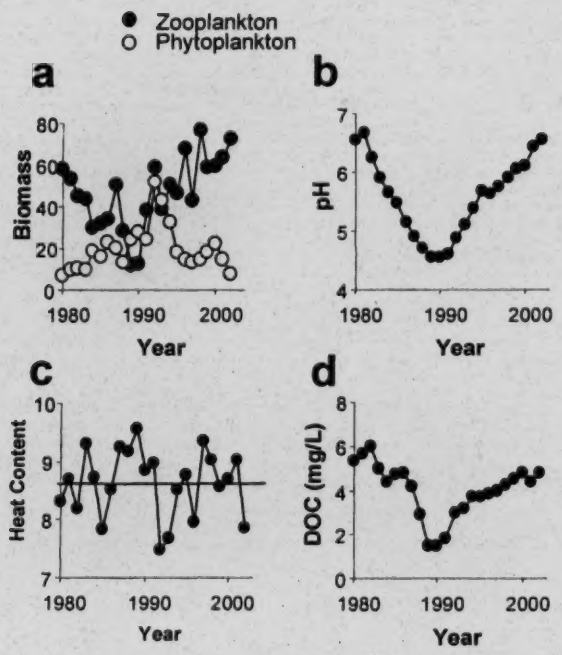

Fig. 3.1. Mean annual changes in the abiotic and biotic variables of the experimentally acidified Lake 302S during ice-free seasons over a 23-yr period. (a) Zooplankton (μg L-1) and phytoplankton (dg m-3) biomass. (b) Surface water pH. (c) Heat content (tera-calories); the 23-yr mean is represented by the horizontal line. (d) Dissolved organic carbon (DOC) concentration (mg L-1).

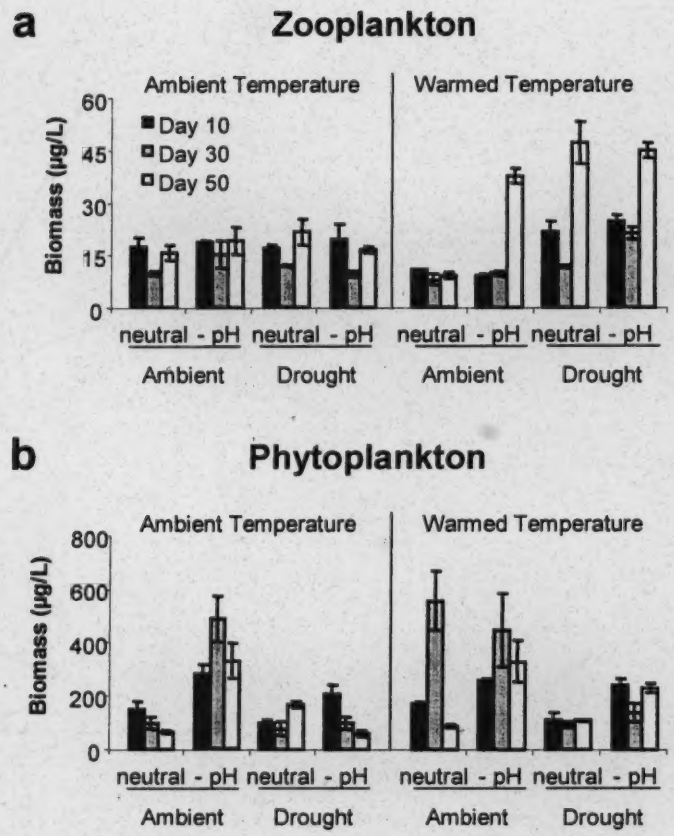

Fig. 3.2. Interactive and direct effects of warming, drought, and acidification on total biomass of (a) planktonic consumers and (b) producers during a 50-day mesocosm experiment. Error bars denote ± 1 SE (n = 3).

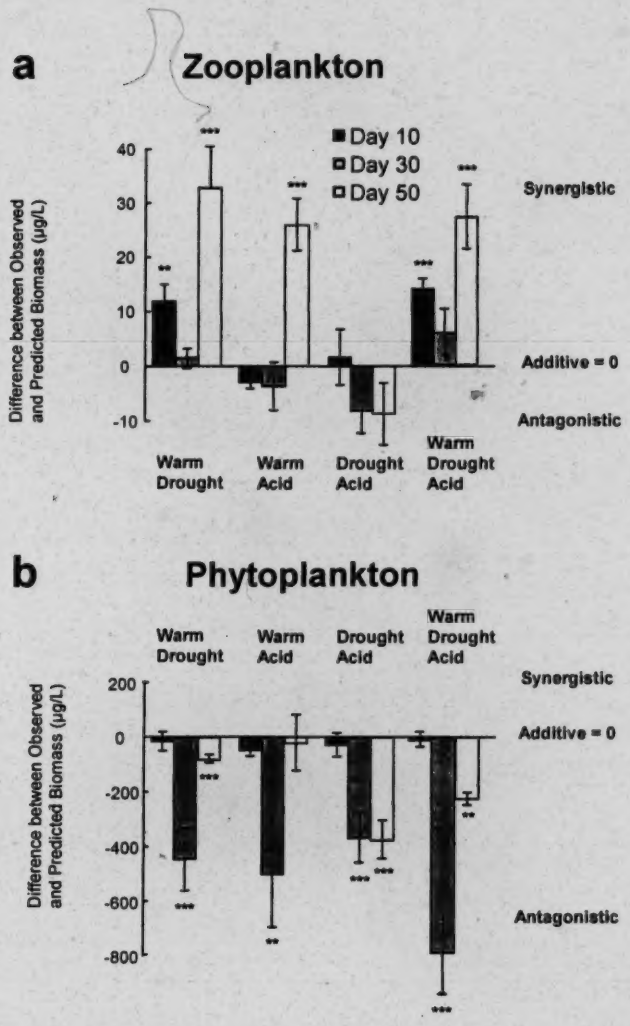

Fig. 3.3. The nature (synergy versus antagonism) and magnitude of the observed interactive impacts of warming, drought, and acidification on total planktonic (a) consumer and (b) producer biomass compared to the sum of their individual effects (i.e. the additive model). Error bars denote ± SE (n = 3 for the 3-way interaction; n = 6 for all 2-way interactions). ** $P < 0.05$, *** $P < 0.01$.

CHAPTER FOUR

GENERAL CONCLUSIONS AND FUTURE RESEARCH

General Conclusions

Consistent with expectations, planktonic communities from both the mesocosm experiment and the history of Lake 302S were highly influenced by the interactive effects among temperature, DOC and pH. The fact that similar responses occurred at two spatial/temporal scales gives high credibility to the fact that interactions (could) occur in lakes across the boreal shield. Contrary to my hypothesis, higher trophic levels did not display more detrimental response to multiple stressors then lower trophic levels. Rather the response to multiple stressors seemed to be species-specific, and could not be related to trophic level, functional grouping or stress tolerance.

Comparison of diversity and function

Given that individuals from lower trophic levels appear highly capable of compensating for the loss of their conspecifics, (Ives 1999; Vinebrooke et al. 2003), and that diversity is typically a more sensitive of an indicator of perturbation than function (if one subscribes to the redundancy hypothesis; Lawton and Brown 1993), I predicted that my experimental manipulations would result in dramatic changes in diversity and composition, but that community biomass would remain largely unaltered. Additionally, I hypothesized that zooplankton rather then phytoplankton communities would demonstrate greater impacts due to their lower diversity and increased susceptibility to stressors (Vinebrooke et al. 2003; Raffaelli et al. 2004). Surprisingly, multiple stressors had little impact on diversity but had large effects on community composition (Chapter 2) and biomass (Chapter 3) for both phytoplankton and zooplankton. I propose that species richness was not reduced due the short timescale of the

experiment, such that 'sensitive' species were substantially reduced, and given enough time, would have been eliminated from the enclosures.

Future Research

Future investigations into the interactions among temperature, DOC and pH on boreal aquatic ecosystems should seek to confirm or disprove the validity that these non-additive effects do occur at the ecosystem level. I believe that similar analyses could be run on comparable systems, for example: Little Rock Lake, Wisconsin (Frost et al. 1999); Swan Lake and the other Sudbury area lakes, Ontario (Yan et al. 1996; Yan et al. 2004); and the Dorset lakes, Ontario (Arnott et al. 2003) as each possess sufficient long-term data, and have been exposed to the combined impacts of warming, DOC reduction and acidification. I hypothesize that these systems would reveal similar results, and would provide strong confirmation to these results and prove that interactions among climate and acidification impact lakes at large geographic scales.

The prevalence of interactions among temperature, DOC and pH suggests that other anthropogenic stressors such as nutrients, metals, invasive species, and toxins may also be highly interactive within lakes. Additionally, anthropogenic impacts may also interact with natural occurrences such as predation (Relyea and Mills 2001), disease (Lafferty and Holt 2003), and disturbances such as forest fires (Bayley et al. 1992), and hydrologic events (Paine et al. 1998; Vieira et al. 2004). More work at both the mesocosm and ecosystem scale is required to identify and characterize these non-additive effects. Further, the study of interactive effects is largely unexplored in the terrestrial landscape, where increases of CO_2, O_3, UV, temperature, disturbances, invasive species and variable precipitation co-occur over wide geographical areas (Aber et al. 2001). The non-additive nature of stressors is becoming increasingly apparent, as evidenced by the increasing number of recent papers exploring this issue (e.g. Doyle et al. 2005; Henry et al. 2005;

Persaud et al 2005; Przeslawski et al. 2005). I propose that interactions amongst multiple stressors are emerging as important issues amid ecologists, and I expect to see this field develop considerably in the next several years.

Scale is an important consideration when investigating the impacts of multiple stressors. Unfortunately, a large number of studies have relied upon relatively small, short-term mesocosm or laboratory studies (e.g. Relyea and Mills 2001; Hoffman et al. 2003; Chen et al. 2004; Rohr et al. 2004; Doyle et al. 2005; Persaud et al 2005; Preslawski et al. 2005 etc. but see Wiegner et al. 2003; Klein et al. 2004; Henry et al. 2005). These small-scale approaches are valid and informative; however, they limit the spatial requirement of motile species and can not assess the impacts of stressors at a variety of life and population stages (Hanazato and Hirokawa 2004). Additionally, small-scale experiments fail to incorporate essential ecosystem-scale processes (Carpenter et al. 1995; Schindler 1998). By combining ecosystem data with larger mesocosms we minimized this degree of artificiality. More realistic experiments mimicking multiple stressors need to be performed if accurate estimations of future global change are to be formed.

One drawback to my mesocosm study is that I can not determine if the response of a single species is due to abiotic conditions, abundance of prey or competition with competitors. For several 'key' species, it is important to determine if they can persist under multiple stressor conditions. For example, *Bosmina* appeared intolerant of warming but competition with *Daphnia* (which favoured warming) may have been more important. As *Bosmina* is regarded as an acid tolerant species, comprising up to 99% of crustacean biomass in acidified lakes (Yan and Strus 1980), discovering intolerance to multiple interactive stressors suggests that zooplankton communities in these lake may be radically altered. Lab-based studies are required to gain insight into the ecology of these key species and their responses to multiple stressors (e.g. Hanazato and Dodson 1995; Williamson et al. 2002).

Lastly, a consensus needs to be reached regarding the use of terminology and statistics in the multiple stressor literature. In particular, the incorrect use of ANOVA in determining the significance of interactions and main effects in multi-factor experiments is distressing as flawed conclusions have been reported within the literature. Additionally, researchers need increased awareness that many experiments are directly testing the interactive effects of multiple stressors, but due to a different focus and keywords, these papers are going unnoticed by those who are reviewing the literature. Chapter one has attempted to address and hopefully resolve both of these issues.

Areas requiring further explanation

From this experiment, a couple of areas of research were never fully explored, such as bacterial and picophytoplankton abundance. Results from the mesocosm experiment suggest that bacterial abundance was generally unresponsive to treatments, and especially interactive effects. Bacterial communities were either generally resilient to abiotic change, or errors associated with determining bacterial abundance were too large and masked treatment effects. Eukaryotic picophytoplankton abundance was only enumerated for the last date, and was either 'high' (> 40,000 cells/ml) or 'low' (< 10,000 cells/ml), never in-between. All of the control bags were 'low', and all the bags subject to *both* warming and acidification had 'high' abundances. However, only 1/3 of the bags that were exposed to only warming, or only acidification also had 'high' abundances. This suggests that warming and acidification help promote an alternative state of 'high' picoplankton abundance, but the combination of the two stressors guarantees this establishment. These results are somewhat expected, given that Stockner and Shortreed (1991) found that increased temperature and low pH are key requirements for eukaryotic picoplankton. There did not appear to be any obvious changes in either the phytoplankton or zooplankton communities as a result of high picoplankton

abundance, thus this result has seemingly questionable ecological relevance and was not considered further. However, I do suggest that a four-fold increase in picoplankton merits additional investigation.

Stoichiometric analyses provided an opportunity for an undergraduate thesis (Michael Clarke; 2005) and were performed on sestonic samples from the mesocosm experiment. It was hypothesized that warming, and increased light penetration caused by DOC removal and acidification would raise sestonic C:P, providing a poorer quality food source for zooplankton, potentially inhibiting *Daphnia* populations (Urabe et al. 2002). Warming moderately increased sestonic C:P, but this was attributable to an effect on a single sampling date. Nutrient ratios were not significantly influenced by DOC removal, acidification, nor treatment combinations. Regardless, high abundance of *Daphnia* and total zooplankton occurred under both high (~500) and low (~300) C:P ratios; the latter has been hypothesized to be the critical limit for *Daphnia*. In summary, stoichiometry (in terms of C:P ratios) does not appear to be a significant factor within the mesocosm experiment. I suggest that further understanding is required of environmental determinants of C:P ratios and how this parameter can affect higher trophic levels before we can begin to predict that impacts of multiple stressors.

Lastly, the mesocosm experiment involved a sub-experiment consisting of short-term grazer inclusion/exclusion containers. Their purpose was to determine if the biotic responses within the mesocosms were largely driven by bottom-up or top-down forces. Due to the prevalence of significant results from the mesocosm experiement, time constraints, and questionable relevance, these cube-container experiments were never analyzed.

Final Considerations

One issue that has continued to surprise me was the continued confirmation of the Lake 302S data by my mesocosm results. Obviously some

overlap is to be expected as they are the same system, but finding support for co-tolerances and tradeoffs among temperature and pH using both methods was interesting. Placing my mesocosm experiment within the context of an ecosystem experiment has, in my mind, greatly increased the believability and application of this thesis. Ecosystem experiments and long-term monitoring are not only highly useful for their own purposes, but also for confirming and validating the results from mesocosm studies decades later. These experimental and reference systems are of immeasurable value and more effort and resources should be used to increase their numbers and scope.

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Appendix A. **Taxonomic composition of zooplankton taxa present in the mesocosm experiment.**

Taxon	Taxon
Cladocerans	**Rotifers**
Alona sp (?)	*Ascomorpha sp*
Bosmina longirostris	*Collotheca mutabilis*
Cerodaphnia/Symphochephus?	*Collotheca pelagica (?)*
Chydorus sphaericus	*Conochilus unicornis*
Daphnia retrocurva	*Filinia longisetae*
Daphnia dubia	*Gastropus hyptopus*
Daphnia mendotae	*Gastropus stylifer*
Daphnia catawba	*Kellicottia bostoniensis*
Daphnia longiremis	*Kellicottia longspina*
Daphnia males	*Keratella cochlearis*
Daphnia neonates	*Keratella taurocephala*
Diaphanosoma birgei	*Lecane sp.*
Eubosmina tubicen	*Polyarthra dolichoptera*
Holopedium gibberum	*Trichocerca cylindrica*
	Trichocerca rousseleti
Copepods	Unknown rotifer sp 1
Calanoid males	Unknown rotifer sp 2
Calanoid Nauplii	Unknown rotifer sp 3
Cyclopoid males	Unknown rotifer sp 4
Cyclopoid Nauplii	Unknown rotifer sp 5
Cyclops sp	Unknown rotifer sp 6
Diaptomus sp	Unknown rotifer sp 7
Epischura lacustris	Unknown rotifer sp 8
Eucyclops sp.	
Mesoscyclops edax	**Other**
Tropocyclops prasinus	*Chaoborus sp*

Appendix B. Taxonomic composition of phytoplankton taxa present in the mesocosm experiment (page 1 of 2).

Taxon	Taxon
Cyanophyceae (Cyanobacteria)	*Staurodesmus sp. 7*
Anabaena sp	*Staurodesmus sp. 8*
Chroccocus minutus	*Staurodesmus sp. 9*
Gompospaeria sp	*Staurodesmus sp. 10*
Lyngbia sp	*Staurodesmus sp. 11*
Merismopedia sp	*Staurodesmus sp. 12*
Merismopedia sp large	*Staurodesmus sp. 13*
Nostoc sp	*Staurodesmus setigerum (?)*
Oscillatoria sp	*Pleurotanium sp.*
Rhabdoderma sp	*Telingia sp.*
Spirulina sp.	*Xanthidium sp.*
Chlorophycae (Green Algae)	**Chrysophyceae (Chrysophytes)**
Ankistrodesmus sp	*Bitrichia chodatii*
Chlorogonium sp (?)	*Chysochromilina sp.*
Coleastrum sp.	*Chrysopherella sp?*
Crucingeniella quadratta	*Chromilina sp*
Crucingeniella rectangularis	*Dinobryon crenulatum*
Crucingenela irregularis	*Dinobryon bavericum Imhof*
Mougeotia sp. (small)	*Dinobryon bavericum variety b*
Mougeotia sp. (large)	*Dinobryon divergens*
Oocystis sp.	*Dinobryon sp. 1*
Pediastrum tetras	*Dinobryon sp. 2*
Quadrigula sp.	*Dinobryon sp. 3*
Scenedesmus sp. 1	large chrysophyte sp.
Scenedesmus sp. 2	*Mallomonas sp.*
Stigogloea sp. (?)	*Mallomonas sp. 2*
Tetradron minimum	*Mallomonas sp. 3*
Zygenema sp.	*Mallomonas sp. 4*
	Mallomonas sp. 5
Desmidaceae (Desmids)	*Mallomonas hetertrichia*
Arthrodesmus sp.	*Mallomonas duerrschmidtiae*
Arthrodesmus triangularis (?)	*Ochromonas sp.*
Cosmarium sp. 1	*Pseudokephrion sp.*
Cosmarium sp. 2	*Salpingoeca sp.*
Euastrum denticulatum (?)	small chrysophyte sp.
Staurodesmus curvatum	*Synura sp.*
Staurodesmus sp. 2	unknown Chrysophyte sp.
Staurodesmus sp. 3	
Staurodesmus sp. 4	
Staurodesmus sp. 5	
Staurodesmus sp. 6	

Appendix B. Taxonomic composition of phytoplankton taxa present in the mesocosm experiment (page 2 of 2).

Taxon
Bacillariophyceae (Diatoms)
Asterionella sp.
Rhizoselinia longiseta
Synedra sp.
Syndera sp. 2
Tabellaria fenestra
Tabellaria floculosa
Pyrrophyceae (Dinoflagelates)
Gymnodinium sp.
Peridinium inconspicum
Peridinium limbatum
Peridinium oinotum/volzii ?
Peridinium wisconsiense
unidentified Dinoflagelate
Unknown species
unknown colony sp.
unknown sp. 1
unknown sp. 2
unknown sp. 3
unknown sp. 5
unknown sp. 6
unknown sp. 7
unknown sp. 9
unknown sp. 10
unknown sp. 11
unknown sp. 12
unknown sp. 13
unknown sp. 14
unknown sp. 15
unknown sp. 16
unknown sp. 17
unknown sp. 18
cryptophyte sp.
cryptophyte sp. 2